NOCoV2 - An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen

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Title Page

Protocol Title:	**NOCoV2 - An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen**
Short Title:	An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301+SOC vs SOC in hospitalized participants with COVID-19 requiring supplemental oxygen
Compound:	RESP301
Indication:	Mild to moderate COVID-19
Study Sponsor:	30 Respiratory PPD London, W1K 6PL, United Kingdom
Protocol Number.:	RESP301-002
Study Phase:	Phase 2/Phase 3
Regulatory Agency Identifying Number:	IND No: Pending EudraCT No: 2020-002120-37
Approval Date:	Final, 01 May 2020

Sponsor Signatory:

PPD

01 May 2020

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Date

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1 Protocol Summary

1.1 Synopsis

Protocol Title: NOCoV2 - An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen

Sponsor Protocol No.: RESP301-002

Study Phase: Phase 2/Phase 3

Sponsor: 30 Respiratory

Rationale:

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design. Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer 3 times a day (TID) for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 4 in the modified World Health Organization (WHO) ordinal scale into levels >4	• Proportion of participants who progress to level >4 of modified WHO ordinal scale due to COVID-19 by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO2	• Change in room air SpO2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time

Objectives	Endpoints
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement to a lower level (<4) of modified WHO ordinal scale • Time to progression to a higher level (>4) of modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	

• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements
	• Physical examinations
	• Vital signs
	• Concomitant medications
	• Cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

Overall Design:

- Open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design in hospitalized patients with COVID-19 requiring supplemental oxygen.

- Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as detailed in Section 6.3.

- The study treatment will be permanently discontinued prior to 10 days if the participant:

    - Improves to level 1 or 2 of the modified WHO ordinal scale;
    - Progresses to a level > 4 of the modified WHO ordinal scale;
    - Experiences adverse drug reaction or suspected adverse drug reaction.

- An Independent Data Monitoring Committee (IDMC) will be responsible for closely reviewing the safety and efficacy data from interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

Disclosure Statement: This is a parallel group treatment study with two arms that are open-label.

Number of Participants:

Approximately 300 participants will be randomly assigned to study intervention (200 to the Investigational arm and 100 to the Control arm).

Intervention Groups and Duration:

The total study duration for a participant from screening to last follow up will be up to 30 days. The study will be divided into the following periods:

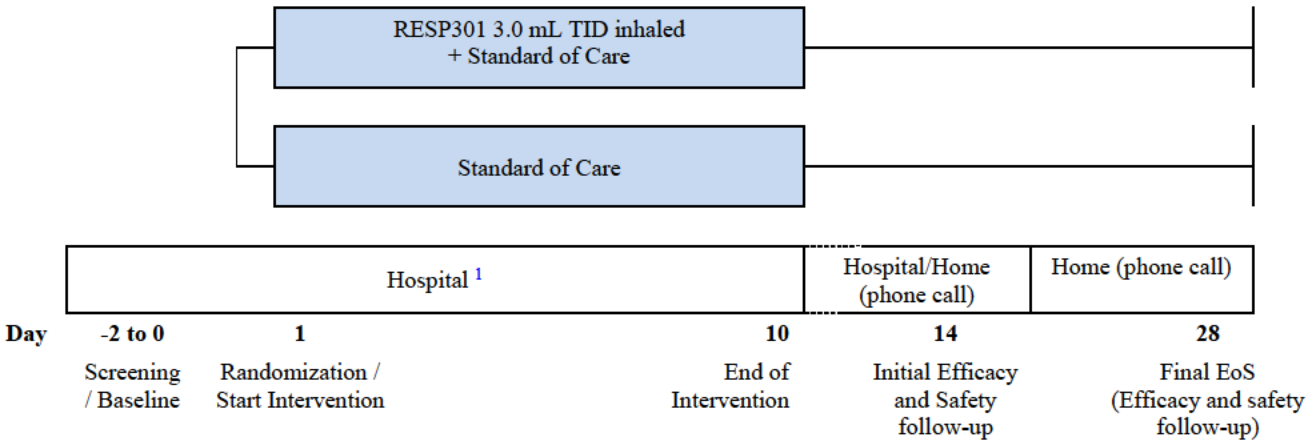
- Screening period: up to 2 days (48 hours) prior to the day of treatment initiation; Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±1 day)

Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer TID for up to 10 days in addition to the SOC while participants in the Control arm will receive SOC alone.

Data Monitoring Committee: Yes

1.2 Schema

Figure 1–1: Study Design



EoS=End of Study; TID=3 times daily

1. Participants may be discharged from hospital before Day 10.

1.3 Schedule of Activities (SoA)

Table 1-1 Schedule of Activities (SoA)

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 1 day)	EoS efficacy and safety follow-up (Day 28) [2] (± 1 day)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Informed consent	X							
Confirmation of COVID-19 infection by nasopharyngeal RT-PCR [3]	X							
Inclusion and exclusion criteria	X	X						Recheck clinical status before 1st dose of study intervention
Demographics	X							
Physical examination including height and weight	X			X	X		X [2]	Full physical exam and height only at screening
Medical history (includes substance usage)	X							History of substance usage or abuse to be recorded but is not exclusionary
Medication history	X							In the prior 3 months
TB screening	X							Serum TB test as per local site
Laboratory assessments (including liver chemistries) [4]	X	X	X [4]	X	X		X [2]	
12-lead ECG	X	X	X	X	X		X	After resting supine for approximately 5 minutes. QRS, QT, QTcF, PR, RR, rate
Vital signs [5]	X	X	X [5]	X	X		X	Within 15 minutes prior to study intervention administration

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 1 day)	EoS efficacy and safety follow-up (Day 28) [2] (± 1 day)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Randomization		X						
Study intervention, TID [6]		X	X	X				Where possible study intervention should be administered at approximately the same times each day At least 4 hours between 2 consecutive inhalations
AE/SAE review		X	X	X	X	X	X	
Device deficiencies check		X	X	X				
Concomitant medication review	X	X	X	X	X	X	X	
Oxygen / respiratory assessment [7]	X	X	X	X				
SpO_2 on room air (after at least 1 minute) prior to study intervention administration and immediately at the end of nebulization [6]		X	X	X				See Section 8.1.2
Modified WHO ordinal scale	X	X	X	X		X	X	See Section 8.1.1
NEWS 2 assessment [7]		X	X	X				Prior to study intervention in the morning. See Section 8.1.3

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 1 day)	EoS efficacy and safety follow-up (Day 28) [2] (± 1 day)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				

Abbreviations: AE=adverse event; aPTT= activated partial thromboplastin time; CBC=complete blood count; Chem 7=blood chemistry panel (sodium, potassium, chloride, bicarbonate/CO_2, blood urea nitrogen, creatinine, glucose); CV=cardiovascular; ECG=electrocardiogram; LFTs=liver function tests; mHb=methemoglobin; PT/INR=prothrombin time/international normalized ratio; RT-PCR=reverse transcription-polymerase chain reaction; SAE=serious adverse event; TB=tuberculosis; TID=three times daily.

1. Screening and randomization can be the same day, providing all eligibility criteria are met.
2. Safety laboratory tests and ECG at follow-up to be collected for participants in hospital at EoS only. For participants who are already discharged at follow-up, there will be a phone call to check AEs/SAEs, concomitant medications, and modified WHO ordinal scale.
3. COVID-19 infection must be confirmed and documented in chart with a positive result on a validated test.
4. Safety: CBC, chem 7; Screening: CBC, chem 7, mHb, LFTs, coagulation (PT/INR, aPTT). Laboratory tests to be repeated as per SOC during treatment period.
5. Vital signs: temperature, heart rate, blood pressure, respiratory rate. Vital signs to be repeated as per SOC during treatment period.
6. Study intervention comprises the following procedures which are to be done in order and as quickly as possible:
   a. Recording of oxygen flow level including mode (nasal cannula, mask)
   b. Recording of SpO_2 and heart rate
   c. Discontinuation of supplemental oxygen administration *
   d. Close participant oversight by trained health care professional until SpO_2 is stable on ambient air after one minute at least *
   e. Recording of SpO_2 * pre-nebulization on ambient air
   f. Preparation of the admixture of RESP301*
   g. Administration of the nebulization via Philips InnoSpire Go nebulizer while monitoring SpO_2 and heart rate (alarms set to 89% and 120 beats / min, or as indicated by the Investigator, for stopping study intervention administration and resuming supplemental oxygen) *
   h. Discontinuation of nebulization after 5 minutes or no product left in the device, whichever comes first *
   i. Recording of SpO_2 * post nebulization on ambient air
   j. Resuming supplemental oxygen *
   k. Participant oversight until SpO_2 is stable *
   l. Recording of SpO_2 *

   * *for participants on active treatment arm only*
7. Immediately before participant is discontinued from oxygen for study intervention administration and assessment of SpO_2 at room air

2 Introduction

2.1 Background

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by a novel coronavirus (SARS-CoV-2). COVID-19 was first described in Wuhan, China, in December 2019 and is now a global pandemic (Matos et al, 2020). Most of those affected have milder illness (80%), 15% will be severely ill (require oxygen) and 5% will require intensive care unit care (Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Of those who are critically ill, most require early intubation and mechanical ventilation. Other complications include septic shock and multi-organ failure, including acute kidney injury and cardiac injury (Yang et al, 2020). Older age and comorbid diseases, such as chronic obstructive pulmonary disease (COPD), hypertension, and diabetes, increase risk of death (Huang et al, 2020; Zhou et al, 2020). The virus is highly contagious and spread via respiratory droplets, direct contact and, if aerosolized, airborne routes. The most common symptoms include fever, fatigue, dry cough, and shortness of breath.

A critical early component of innate host defense in the airway is the ability of respiratory epithelial cells to produce high levels of NO (Kao et al, 2001). NO functions as a signaling molecule in initiation of the inflammatory response to viruses, and also has direct antiviral effects (Folkerts et al, 1998). The airway epithelium has highly efficient nitric oxide (NO) synthetic machinery which is amplified in viral infection. Healthy human airway epithelium has abundant expression of the endothelial enzyme NOS II due to continuous transcriptional activation of the gene in vivo. Loss of NO synthesis in lung diseases predisposes individuals to increased virus/microbe infection (Xu et al, 2006).

The physiological roles of nitric oxide (NO) and the enzymatic pathways for its synthesis via NO synthase have been clearly established for many years (Tucker et al, 2007). In particular, NO has been demonstrated to have potent anti-microbial properties against a wide range of pathogens. An alternative non-enzymatic synthetic pathway for NO synthesis has been developed, which generates NO and related higher oxides of nitrogen (NOx) via the chemical reactions of acidified nitrite (Hardwick et al, 2001, Tucker et al, 1999). A solution of co-mixed NO/NOx and ascorbic acid (RespiNOS), delivered by nebulizer, was found to be safe and well tolerated in healthy volunteers (Tucker et al, 2007). Spectral investigations further confirmed that there were no potentially harmful moieties present in the solution. The study concluded that RespiNOS had potential for use as a broad-spectrum anti-microbial agent in patients with chronic bronchial sepsis such as bronchiectasis, COPD, and cystic fibrosis.

RESP301 is a NO-generating liquid designed to release NO in situ in the upper airways and deep in the alveolar spaces. RESP301 is delivered via a handheld nebulizer and has specific advantages (see below) over inhaled NO gas in treating patients with COVID-19 during the current pandemic. It has shown high in vitro activity against respiratory pathogens, both viral and bacterial.

Key advantages of RESP301 over inhaled NO gas are:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

The Sponsor has conducted a number of supportive in vitro studies to assess the activity of NO-generating solutions against major respiratory viruses responsible for infections in humans. These studies include the following:

- Preliminary in vitro study to demonstrate the effects of RESP301 on SARS-CoV-2 replication (Study RP026a)
- In vitro study of RESP301 against influenza A (H1N1) and human rhinovirus including in vitro RESP301 cytotoxicity study (Study RP010)
- Determination of the anti-viral efficacy of six NO-releasing formulations against two strains of virus (influenza A virus [H1N1] and human rhinovirus 16) (Study 30 Respiratory 009)
- Evaluation of inhaled nitrite immune effects and antimicrobial activity against intracellular drug sensitive and drug resistant *Mycobacterium tuberculosis* and *M. abscessus* infections (Study RP014)
- RESP301 in combination with antibiotic treatment (Study RP002)
- Anti-microbial activity of acidified nitrite solution against *M. abscessus* (Study TR001)

Preliminary data suggest that incubation with RESP301 for 48 hours inhibits replication of SARS-CoV-2 (Log_{10} TCID 50/mL – 2.1- fold reduction), and reduces the viral load to levels below the limit of detection of the assay, at concentrations which were not associated with cytotoxicity (Study RP026a).

Studies of inhaled nebulized sodium nitrite (AIR001) in healthy subjects and in patients showed that inhaled acidified nitrite produces dose proportional plasma pharmacokinetics without

accumulation following repeated administration, with low systemic blood levels of nitrite and methemoglobin (<3%) (Rix et al, 2015). Inhaled nitrite (AIR001) at doses up to 90 mg three times daily (TID) displayed a good safety profile and is well tolerated (Parsley et al, 2013; Simon et al, 2016), thus supporting the investigation of RESP301 in the clinical setting.

RESP301 is an admix solution of two precursor solutions mixed together at point of care for immediate inhaled administration via a nebulizer device (a hand-held CE marked Philips InnoSpire Go vibrating mesh nebulizer, Food and Drug Administration [FDA]-approved and European CE marked). The silent mesh nebulizer is used for the treatment of children and adults with pulmonary diseases. The InnoSpire nebulizer generates aerosols by vibrating to draw liquid medication through approximately 1000 funnel-shaped apertures (Ari and Fink, 2020) and has been shown to be an effective mesh nebulizer in a small scale experiment intended to test new in-process controls minimizing variability in aerosol performance (Hatley et al, 2016). The device is small, portable, easy to use, and cleaning and maintenance is simple due to the simplicity of the device (Ari and Fink, 2020).

A detailed description of the chemistry, pharmacology, efficacy, and safety of RESP301 is provided in the Investigator's Brochure.

2.2 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of RESP301 may be found in the Investigator's Brochure.

2.2.1 Risk Assessment

Table 2-1 Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention RESP301		
Participants need to be discontinued from supplemental oxygen for up to 4 minutes to receive nebulized study treatment	Study treatment is to be administered via a hand-held mesh nebulizer (Philips InnoSpire Go) which is designed for oral inhalation. In addition, as NO spontaneously reacts with O_2	Participants unable to be safely discontinued from supplemental oxygen will be excluded (Exclusion criterion 2)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	to produce NO_2 (a bronchial irritant), it is potentially unsafe to use both treatments concomitantly	Treatment administration is to be performed as quickly as possible (Section 1.3) Oxygen saturation (SpO_2) and heart rate will be monitored throughout the study intervention administration (Section 1.3) Stopping rules to resume supplemental O_2 in case of decrease in SpO_2 or clinical signs of intolerance (Section 7.1)
Risk of methemoglobinemia	Very low levels are produced but as methemoglobinemia has been reported with continuous NO gas administration, the risk cannot be firmly ruled out	Participants with a history of methemoglobinemia will be excluded (Exclusion criterion 4) and methemoglobin (mHb) is included in safety laboratory tests (Section 1.3)
Risk of clinical bronchial hyper-responsiveness related to nebulization	Excipients in the nebulized RESP301 include mannitol which is a known bronchial irritant and may potentially cause or exacerbate cough or bronchospasm	Participants with a known history of moderate or severe bronchial hyperreactivity (such as in asthma) or presence of signs of significant bronchospasm on examination will be excluded from study participation (Exclusion criterion 5)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Nebulization	The delivered dose of RESP301, estimated to be ~40% of the masses listed in Table 6-2, can be affected by patient technique and inspiratory capacity leading to inter- and intra-patient variation in actual doses of these ingredients, given the acute nature and high disease burden in this proposed clinical setting	Training will be provided to study staff to minimize variation in dose
Other		
Risk of spreading SARS-CoV-2	SARS-CoV-2 is known to be transmissable via respiratory droplets and any interventions that may potentially increase cough or aerosolization of respiratory secretions of an infected patient may increase risk of spread of disease	Proper personal protective equipment including appropriate mask, face shield, gown and gloves should be used at all times. When possible, the site staff should not remain in the room during study intervention administration (providing that continuous O_2 monitoring can be conducted remotely and study intervention can be administered correctly)

2.2.2 Benefit Assessment

To date, no treatment of COVID-19 has demonstrated clinical efficacy. Patients and their physicians are critically in need for treatments that decrease the risk of severe levels of disease,

particularly the rate of intubation or other ventilatory support as they significantly lead to fatal outcomes. Considering the current pandemic, even a limited improvement in the rate of progression to severe stage of the disease would provide sizable benefits for patients and society.

Nitric Oxide is already marketed (e.g., INOmax®, NOXIVENT™, etc) in the United States (US) and other countries as a gas for continuous use in preterm and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension in conjunction with ventilatory support and other appropriate agents. It is well tolerated, although under continuous use, a small number of cases of methemoglobinemia have been reported (NOXIVENT™ FDA Prescribing Information).

Nitric oxide has been shown in vitro to inhibit the replication cycle of severe acute respiratory syndrome coronavirus (Akerström S et al, 2005). In addition, NO is a naturally occurring and potent antimicrobial agent in the human body, which is active against viruses, bacteria, fungus, and yeasts (Fang, 1997). In particular, NO inhibits replication in vitro in a number of respiratory viruses including influenza A and B (Rimmelzwaan et al, 1999, Regev-Shoshani et al, 2013), human rhinovirus (Sanders et al, 1998), and respiratory syncytial virus (Ali-Ahmad D et al, 2003).

The ability of RESP301 to have a marked antibacterial action is highly relevant in the context of treating patients with SARS-CoV-2 infection since superimposed bacterial infection is a critical factor and a major cause of morbidity and mortality. RESP301 would restore and replenish the NO deficiency in patients who have succumbed to SARS-CoV-2 infection. RESP301 also has key advantages over inhaled NO gas, as discussed in Section 2.1.

The product will be delivered via a Philips InnoSpire Go nebulizer which is approved for use both in the United States of America (510k Clearance) and in Europe (CE mark). The InnoSpire Go is a hand-held, single patient use, vibrating mesh nebulizer system designed to aerosolize liquid medications for respiratory disease. The device operates continuously once initiated and automatically switches off once the medication has been delivered. The device may be used in pediatric and adult populations, as permitted by the prescribed medication, and is suitable for use in home environments or hospital/clinic settings. It appears to be easily cleaned and delivers a fine mist (particle size < 10 µM) (FDA InnoSpire Go Indications for Use).

As testing inefficient product would distract sites from participating in other research efforts, two interim analysis for futility purpose have been planned.

2.2.3 Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to participants participating in this study, the potential risks identified in association with RESP301 are justified by the anticipated benefits that may be afforded to participants with COVID-19.

3 Objectives and Endpoints

Table 3-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 4 in the modified World Health Organization (WHO) ordinal scale into levels >4	• Proportion of participants who progress to level >4 of modified WHO ordinal scale due to COVID-19 by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO_2	• Change in room air SpO_2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement to a lower level (<4) of modified WHO ordinal scale • Time to progression to a higher level (>4) of modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	
• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements • Physical examinations • Vital signs

Objectives	Endpoints

	• Concomitant medications
	• Cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

4 Study Design

4.1 Overall Design

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study in hospitalized participants with COVID-19 requiring supplemental oxygen (WHO ordinary scale level 4) using a sequential adaptive design. Approximately 300 participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer TID for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

The study will be divided into the following periods:

- Screening period: up to 2 days (48 hours) prior to the day of treatment initiation; Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28

- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±1 day)

After screening, eligible participants will be randomized to either RESP301+SOC or SOC alone on Day 1, and the study treatment will be initiated as applicable (Table 1-1). Screening and randomization may happen on the same day (Day 1).

An individual administration of study intervention will be stopped, and supplemental oxygen resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Section 4.2 and Section 8.1.2). However, the participant may continue with subsequent doses if the investigator judges that the benefit risk remain positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored. Inhalation of study intervention should be completed within 20 minutes.

The study treatment will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale;
- Experiences adverse drug reaction or suspected adverse drug reaction.

In the above cases, participants will be considered as ongoing in the study until the final Safety follow-up phone call (Day 28). Participants will be withdrawn from the study prior to Day 10 (Early withdrawal) only if they withdraw consent.

The total study duration for a participant from screening to last follow up will be up to 30 days.

Participants will be stratified by country and presence of risk factor(s) for severe outcomes of COVID-19, based on comorbidities and age, as detailed in Section 6.3.

Two interim analyses are planned (see Section 9.5 for details).

An Independent Data Monitoring Committee (IDMC) will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

4.2 Scientific Rationale for Study Design

NO is a naturally occurring and potent antimicrobial agent in the human body which has been shown in vitro to inhibit replication in vitro of severe acute respiratory syndrome coronavirus

(Akerström S et al, 2005), including SARS-CoV2, and other respiratory viruses. NO as an inhaled gas is already marketed in the US and other countries (see Section 2.2.2) but has disadvantages over NO produced locally in the oropharynx or lung airways (lengthy treatment requiring NO canisters, the inhaled NO is expelled in exhalation, the NO gas oxidizes in air to form toxic NO_2 which is a potential lung irritant and a contaminant in the patient's environment, and the half-life of NO in air is short).

RESP301 has potential advantages over inhaled NO gas:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

In this study, the effect of RESP301 as an add on treatment to SOC will be evaluated for its efficacy in reducing rate of progression to a more severe level of COVID-19 and for safety by comparison with SOC alone in hospitalized COVID-19 patients. A sequential adaptive design was chosen in order to assess futility and sample size after interim analyses (see Section 9.5). The study design was developed in accordance with the latest WHO and regional regulatory agencies guidelines.

Several risks factors for severe or fatal outcomes have been reported (Bialek et al, 2020; Huang et al, 2020; Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020; Rong-Hui et al, 2020; Zhou et al, 2020). To minimize bias, the results will be stratified according to the known risk factors as described in Section 6.3.

In this study, measures will be in place to trigger discontinuation of study intervention at a SpO_2 limit pre-agreed at the site (see Section 8.1.2). There is no critical level of oxygen saturation below which tissue hypoxia occurs due to the large number of variables that contribute to hypoxia at the tissue and cellular level (temperature, pH, tissue blood flow). As a result, there is no consensus about what constitutes normal and abnormal oximetry (ATS/ACCP, 2003). To support and guide the team in charge of study intervention administration, a default value of SpO_2=89% and heart rate (120 beats/min) has been proposed as a suitable limit of tolerance. However, for the reason stated above, this limit can be overruled by the Investigator in either direction.

Newly released guidelines for ongoing clinical trials during the COVID-19 pandemic have emphasized the need to reduce the burden on sites and clinical trial personnel/investigators whether they be administrative, site visit, or other burdens (ACRO, 2020; EMA, 2020; HRS, 2020). Thus, without blinding and a placebo, along with minimal procedures, the additional burden on clinical staff has been reduced as much as possible.

4.3 Justification for Dose

Studies with nebulized sodium nitrite (AIR001) in healthy subjects and in patients have shown that inhaled nitrite produces dose proportional plasma pharmacokinetic without accumulation following repeated administration, with low systemic blood levels of nitrite and methemoglobin (<3%). Inhaled nitrite at doses up to 90 mg TID displayed a good safety profile and is well tolerated (Rix et al, 2015; Parsley et al, 2013; Simon et al, 2016).

In this study, 6.0 mL RESP301 (delivered dose 62 mg) will be administered via a Philips InnoSpire Go hand-held vibrating mesh nebulizer, TID with at least 4 hours between each dose. Each 6.0mL dose for nebulization contains the masses described in Table 6-2.

4.4 End of Study Definition

A participant is considered to have completed the study if he/she has completed the Treatment Period and the Follow-up Period (through the Day 28 EoS Follow-up).

5 Study Population

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

The study population will consist of participants with COVID-19. Participants must be able to provide written consent and meet all the inclusion criteria and none of the exclusion criteria.

5.1 Study Rationale

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 plus SOC versus SOC alone in hospitalized patients with COVID-19 requiring supplemental oxygen (WHO level 4).

Each constituent (see Section 6.2) of RESP301 is used as SOC for various conditions. Product application is straightforward, requiring a few minutes of inhalation using a standard nebulizer.

RESP301 is likely to be beneficial in treating patients with COVID-19 who are not receiving ventilation but are using supplementary oxygen in order to maintain a safe level of SpO_2.

Considering the well-established and global use of NO and the device, and the current public health emergency resulting from the COVID-19 pandemic, a Phase2/3 study of RESP301 is considered reasonable in order to generate efficacy data in patients infected with COVID-19.

5.2 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant is ≥18 years of age, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participant has laboratory-confirmed SARS-CoV-2 infection as determined by reverse transcriptase polymerase chain reaction (RT-PCR) or other approved clinical testing prior to randomization.
3. Participant is hospitalized in relation to COVID-19, requiring supplemental oxygen to maintain SpO_2 at a safe level (WHO level 4).

Sex

4. Participant is male or female.

Informed Consent

5. Participant is capable of giving signed informed consent as described in Appendix 1 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.3 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Rapidly deteriorating or likely to require escalation to high flow oxygen, invasive or non-invasive ventilatory support within 24 hours according to Investigator's opinion.
2. Unable to safely receive a nebulized treatment while on room air for approximately 4 minutes according to Investigator's opinion.

3. Unable to receive or considered ineligible for invasive or non-invasive ventilatory support.
4. History of methemoglobinemia.
5. Uncontrolled asthma or history of severe bronchospasm.
6. Severe (requiring baseline oxygen therapy > 12 h/day prehospitalization) chronic respiratory disease (e.g., known COPD, pulmonary arterial hypertension, idiopathic pulmonary fibrosis, interstitial lung disease).
7. Suspected or confirmed untreated, active tuberculosis.
8. Severely immune-compromised participants in Investigator's opinion.
9. Recent (within 3 months) active coronary artery disease or decompensated heart failure (New York Heart Association class 3-4).
10. Presence of tracheostomy.

Prior/Concomitant Therapy

11. Chronic (≥4 weeks) use of corticosteroids >10 mg/day of prednisone or equivalent within 4 weeks of randomization.

Prior/Concurrent Clinical Study Experience

12. Participation in other clinical investigations utilizing investigational treatment or within 30 days / 5 half-lives whichever is longer.

Diagnostic Assessments

13. Clinically significant abnormalities in clinical chemistry or hematology at screening, defined as:

- Platelet count <50,000 mm^3
- Alanine aminotransferase or aspartate aminotransferase >5 × upper limit of normal (ULN).
- Estimated glomerular filtration rate <30 mL/min/1.73 m^2 (modification of diet in renal disease formula) or requiring hemofiltration or dialysis.

Other Exclusions

14. Anticipated transfer to another hospital which is not a study site during the treatment period.
15. Allergy to any of the components of the study intervention.

5.4 Lifestyle Considerations

No restrictions are required.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography and reason for screen failure (e.g., eligibility requirements failed).

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

5.5.1 Screening and Enrollment Log and Participant Identification Numbers

The participant's enrollment will be recorded in the Screening and Enrollment Log.

Upon enrollment, each participant will receive a unique participant identification number. Participant numbers must not be re-used for different participants.

6 Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1 Study Intervention(s) Administered

Table 6-1 Study Intervention(s) Administered

Study Treatment	RESP301 + SOC
Intervention Name:	RESP301
Type:	Drug
Dosage Formulation:	Admixture for inhalation
Unit Dose Strength(s):	Delivered dose (62 mg) sodium nitrite ($NaNO_2$) (6 mL of NO-generating admixture containing 150 mM $NaNO_2$, 50 mM mannitol and 100 mM citric acid)
Dosage Level(s):	One 6 mL-inhalation TID with at least 4 hours between 2 consecutive inhalations.
Route of Administration:	Inhalation
IMP and NIMP:	IMP
Sourcing:	RESP301 provided centrally by the Sponsor.
Dosing Instructions:	A CE marked Philips InnoSpire Go hand-held vibrating mesh nebulizer will be used to administer the RESP301, under the direction of the study physician. Treatment is to commence within 5 minutes of RESP301 preparation and each inhalation takes approximately 3-5 minutes. Before initiating each RESP301 inhalation, supplemental oxygen will be interrupted for a few minutes until SpO_2 levels are stable. Depending on the extent of SpO_2 decrease, the RESP301 inhalation may be initiated or supplemental oxygen may be resumed at the discretion of the investigator.
Packaging and Labeling:	Study intervention will be provided as two separate ampoules of $NaNO_2$/mannitol (3 mL) and citric acid buffered to pH 5.4 (3 mL) which will be labeled as required per country requirement.

6.1.1 Medical Devices

1. Medical devices (not manufactured by or for 30 Respiratory) provided for use in this study are FDA approved and CE marked Philips InnoSpire Go hand-held vibrating mesh nebulizer.
2. Instructions for medical device use are provided in the User Manual.

3. All medical device deficiencies, including those resulting from malfunctions of the device, must be detected, documented, and reported by the investigator throughout the study (see Section 8.3.7).

6.1.2 Device Training

Training will be provided to ensure all study staff are familiar with the device, including mixing the solutions, adding the admixture to the device and cleaning the device.

6.2 Preparation, Handling, Storage, and Accountability

6.2.1 Preparation of Study Intervention Product

RESP301 is prepared at bedside by mixing two sterile 3 mL solutions (one of $NaNO_2$/mannitol and one of citric acid buffered to pH 5.4). The solution is to be nebulized immediately or within 5 minutes post mixing as per the instructions given in the SoA (Table 1-1). The first administration of study intervention should be provided under medical supervision.

The unmixed RESP301 drug product consists of a solution of concentrated sodium nitrite with mannitol excipient (3 mL). The citric acid solution (3 mL) is then added to the sodium nitrite/mannitol pre-mixture ampoule and inverted 10 times to mix no more than 5 minutes prior to application into the nebulizer (see Table 6-2 for details of composition). This combined mildly acidified nitrite solution (6 mL) is then inhaled through the mouth using a Philips InnoSpire Go hand-held mesh nebulizer under the direction of a physician. The purpose of the acidified nitrite aerosol is to deliver NO_2 to the participant; each dose of 41.4 mg of nitrite (as the anion) could theoretically deliver 27.0 mg of NO_2, assuming a 100% conversation rate. The amount of NO_2 is not measured directly but inferred from the amount of nitrite available for conversion.

Table 6-2 Composition of Drug Product, Diluent and Admixure

	Material	Function	Quantity per vial
Drug Product	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Diluent	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Acidified Drug Product Mixture	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**6.0 mL**

6.2.2 Storage and Accountability of Study Intervention

The investigator or designee must maintain a log to confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (e.g., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study intervention are provided in the Investigator Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an interactive voice response system (IVRS)/interactive web response system

(IWRS). The site will contact the IVRS/IWRS prior to the start of study intervention administration for each participant. The site will record the intervention assignment on the applicable case report form (CRF), if required.

Potential bias will be reduced using a central stratified randomization to assign participants into one of the study treatment arms, RESP301+SOC or SOC (control), with a randomization ratio of 2:1. Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as follows:

1. Age >65 years
2. Ongoing or currently treated diabetes mellitus
3. Ongoing or currently treated hypertension
4. Ongoing or currently treated cardiovascular disease
5. Ongoing or currently treated chronic lung disease
6. Cancer history of less than 3 years, basal cell skin carcinoma excluded
7. Ongoing or currently treated chronic kidney disease

Participants will be stratified as no risk factor (none of the above criteria), one risk factor (one single of the above) or high-risk factor (2 or more of the above).

6.4 Study Intervention Compliance

Participants will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each inhalation will be recorded in the source documents and recorded in the CRF. The study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Concomitant Therapy

During the study, participants will receive institutional SOC for the treatment of COVID-19.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates

- Dosage information including dose and frequency and route

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.6 Intervention After the End of the Study

After the end of the study, participants may resume or continue their SOC (if any). Study intervention for COVID-19 will not continue beyond this study.

7 Discontinuation of Study Intervention and Participant Discontinuation

7.1 Discontinuation of Study Intervention

It may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the study to be evaluated for the Day 14 and Day 28 safety follow-ups. See the SoA (Table 1-1) for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

Study intervention will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale;
- Experiences adverse drug reaction or suspected adverse drug reaction.

Additionally, study intervention should be discontinued in the following circumstances:

1. Clinical intolerance to nebulization.

   An individual administration of study treatment will be stopped, and supplemental oxygen resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Sections 4.2 and 8.1.2).

2. Discontinuation of study intervention for abnormal liver tests should be considered if the Investigator believes that it is in best interest of the participant.

3. If a clinically significant change in ECG is identified, the Investigator should exercise their clinical judgement to decide whether continuing study intervention administration is in the best interest of safety of the participant.

4. AE/SAE if the Investigator believes that it is in best interest of the participant, including the following:

5. Development of significant broncho spasm or worsening of cough:

- Progressive COVID-19 requiring initiation of non-invasive or invasive ventilation or high flow oxygen
- Development of mHb level above 3%
- Inability to safely continue receiving study intervention due to compromised respiratory status while off supplemental oxygen and receiving nebulized therapy (manifest by drop in SpO_2, increased respiratory rate or clinical other findings)

6. Progression of disease.

Refer to the SoA (Table 1-1) for data to be collected at the time of study intervention discontinuation and follow-up and for any further evaluations that need to be completed.

Brief temporary discontinuation of study intervention is permitted during the study, providing inhalation of study intervention is completed within 20 minutes. The participant may continue with subsequent doses if the investigator judges that the benefit risk remain positive.

7.2 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. The participant will be definitively discontinued from both the study intervention and from the study at that time.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

At the time of discontinuing from the study, if possible, an early discontinuation assessment should be conducted, as shown in the SoA (Table 1-1).

7.3 Loss of Participants to Follow-Up

A participant will be considered lost to follow-up if he or she is unable to be contacted by the study site. The following actions must be taken if a participant cannot be contacted at Day 14 or Day 28:

- The site must attempt to contact the participant and counsel the participant on the importance of maintaining the assigned schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- In cases in which the participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record/CRF.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 Study Assessments and Procedures

Study procedures and their timing are summarized in the SoA (Table 1-1). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Table 1-1).

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 20 mL.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Informed Consent

Informed consent must be documented according to Appendix 1, Section 10.1.3.

Eligibility Criteria

All inclusion and exclusion criteria will be reviewed by the Investigator or designee to ensure that the participant qualifies for the study.

Participant Identification Card

All participants will be given a participant identification card identifying them as participants in a research study. The card will contain study site contact information (including direct telephone numbers) to be used in the event of an emergency. The Investigator or qualified designee will provide the participant with a participant identification card immediately after the participant provides written informed consent. At the time of intervention allocation/randomization, site personnel will add the intervention/randomization number to the participant identification card.

Medical History

A medical history will be obtained by the Investigator or qualified designee. The medical history will collect all active conditions and any condition diagnosed that the Investigator considers to be clinically relevant. Details regarding the disease for which the participant has enrolled in this study will be recorded separately and not listed as medical history.

Prior and Concomitant Medications Review

The Investigator or qualified designee will review prior medication use and record prior medications taken by the participant within 3 months prior to screening. This should include a history of hypertension medication, in particular angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

The Investigator or qualified designee will record medication, if any, taken by the participant during the study through the last assessment. Concomitant medications will be recorded on an ongoing basis during the study (or longer if related to an SAE).

8.1 Efficacy Assessments

8.1.1 Modified WHO Ordinal Scale

A modified WHO ordinal scale will be used for consistency with the recent study of lopinavir-ritonavir in adults hospitalized with severe COVID-19 (Cao et al, 2020), to record the participant's status at the time of assessment. The modified WHO ordinal scale includes the following levels:

1. Not hospitalized, no limitations on activities;
2. Not hospitalized, limitation on activities;
3. Hospitalized, not requiring supplemental oxygen;
4. Hospitalized, requiring supplemental oxygen;
5. Hospitalized, on non-invasive ventilation or high-flow oxygen devices;
6. Hospitalized, on invasive mechanical ventilation or extra corporeal membrane oxygenation (ECMO);
7. Death.

8.1.2 Pulse Oximetry

Pulse oximetry measurements will be performed to evaluate SpO_2 as outlined in the SoA (Table 1-1) and in accordance with the site standard operating procedures, on a medical-grade medical device. Measurements will be taken with a probe on fingertip or earlobe and recorded as percent oxygenated hemoglobin. Supplemental oxygen used at the time of assessment and method of oxygen delivery will be collected and documented along with the SpO_2.

Prior to administration of study intervention, supplemental oxygen will be held for at least a minute and SpO_2 checked in order to ensure participant may safely receive the study intervention. For safety reasons, since due to the current pandemic it cannot be guaranteed that study intervention will be consistently administered under direct medical supervision, the study Investigators should provide study personnel who are able to monitor the participant and their SpO_2 and ensure that should it decline to a threshold, this would trigger immediate discontinuation. By default, unless overruled by the study Investigator, this SpO_2 threshold is set to 89%. This threshold should be entered into the saturation monitoring device to trigger an alarm during nebulization.

FiO_2 will be measured per site standard practice and recorded in the eCRF. The device used to administer oxygen should also be recorded.

8.1.3 National Early Warning Score (NEWS) 2

The NEWS 2 will be measured prior to study intervention in the morning according to the SoA in Table 1-1.

The NEWS is based on a simple aggregate scoring system in which a score is allocated to physiological measurements, already recorded in routine practice, when patients present to, or are being monitored in hospital (RCP, 2012). Six simple physiological parameters form the basis of the scoring system:

1. Respiration rate
2. Oxygen saturation
3. Systolic blood pressure
4. Pulse rate
5. Level of consciousness or new confusion*
6. Temperature

**The patient has new-onset confusion, disorientation and/or agitation, where previously their mental state was normal – this may be subtle. The patient may respond to questions coherently, but there is some confusion, disorientation and/or agitation. This would score 3 or 4 on the Glasgow Coma Scale (rather than the normal 5 for verbal response), and scores 3 on the NEWS system.*

A score is allocated to each parameter as they are measured, with the magnitude of the score reflecting how extremely the parameter varies from the norm (zero for 'normal'; maximum score 3). The score is then aggregated. The score is increased by 2 points for people requiring supplemental oxygen to maintain their recommended oxygen saturation. This is a pragmatic approach, with a key emphasis on system-wide standardization and the use of physiological parameters that are already routinely measured in National Health Service (NHS) hospitals and in prehospital care, recorded on a standardized clinical chart – the NEWS 2 chart (Refer to Appendix 2: The NEWS 2 Scoring System).

Reproduced from: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017 (RCP, 2017).

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1-1).

8.2.1 Physical Examinations

A complete physical examination will include, at a minimum, assessments of the head/ear/eyes/nose/throat, cardiovascular, respiratory, gastrointestinal, lymphatic, skin and neurological systems. Height (screening only) and weight will also be measured and recorded.

A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

8.2.2 Vital Signs

Vital signs to be collected as outlined in the SoA (Table 1-1) and include body temperature, heart rate, blood pressure, and respiratory rate.

Body temperature will be assessed per the local practice (temporal or otic are preferred sites), and site will be recorded. Pulse rate, respiratory rate, and blood pressure will also be assessed per site SOC. Where possible the same methods should be used throughout the study for an individual participant.

Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones). During study intervention administration, an alarm threshold should be set for heart rate at 120 beats/min (or as indicated by the Investigator), as outlined in the SoA (Table 1-1).

Vital signs (to be taken before blood collection for laboratory tests) will consist of one pulse and one blood pressure measurement (after the participant has been sitting for 5 minutes).

8.2.3 Electrocardiograms

Single 12-lead ECG will be obtained as outlined in the SoA (Table 1-1) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals for local reading.

8.2.4 Clinical Safety Laboratory Assessments

Refer to Appendix 3 for the list of clinical laboratory tests to be performed and to the SoA (Table 1-1) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically relevant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically abnormal during participation in the study or within 4 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, where possible, and the Sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the laboratory manual and the SoA (Table 1-1).
- If laboratory values from laboratory assessments not specified in the protocol and performed at the institution's local laboratory result in the need for a change in participant management or are considered clinically relevant by the Investigator (e.g., are considered to be an SAE or an AE or require dose modification), then the results must be recorded in the CRF.

8.3 Adverse Events and Serious Adverse Events

The definitions of AEs and SAEs can be found in Appendix 4.

Table 8-1 summarizes the categories of AEs.

Table 8-1 Categories of Adverse Event

	Non-device related	**Device- or procedure-related**	
Non-Serious	AE (includes all categories)	ADE	
Serious	SAE (includes all categories that are serious)	SADE	
		Anticipated	**Unanticipated**
		ASADE	USADE

Abbreviations: ADE=adverse device effect; AE=adverse event; ASADE=anticipated serious adverse device effect; SADE=serious adverse device effect; SAE=serious adverse event; USADE=unanticipated serious adverse device effect

The definitions of AEs and SAEs (including adverse device effects [ADEs], serious ADEs [SADEs], and unanticipated SADEs [USADEs]), can be found in Appendix 5. Device deficiencies are covered in Section 8.3.8.

Adverse events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AE that are serious, considered related to the study intervention or the study, or that caused the participant to discontinue the study intervention (see Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from randomization until the last follow-up visit at the time points specified in the SoA (Table 1-1).

Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the CRF not the AE section.

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours, as indicated in Appendix 4. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obliged to actively seek AEs or SAEs after the conclusion of study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.3.2 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 4.

8.3.4 Regulatory Reporting Requirements for SAE

Prompt notification (within 24 hours, see Appendix 4) by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and Investigators.

For all studies except those utilizing medical devices, Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAE) from the Sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy (Not Applicable)

Women of childbearing potential and pregnant women are allowed in the study.

8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related events (DREs) are common in participants with COVID-19 and can be serious/life-threatening:

- Fever
- Cough
- Dyspnea*

- Asthenia

** A cough or dyspnea episode related to study intervention administration does not meet the definition of a DRE and should be reported as an AE.*

Because these events are typically associated with the disease under study, they will not be reported as AEs.

NOTE: However, if either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the Investigator's opinion, of greater intensity, frequency, or duration than expected for the individual participant.

OR

- The Investigator considers that there is a reasonable possibility that the event was related to study intervention.

8.3.7 Adverse Events of Special Interest (Not Applicable)

No AEs of special interest are defined for this study.

8.3.8 Medical Device Deficiencies

The definition of a medical device deficiency and instructions for documenting medical device deficiencies are provided in Appendix 5.

Medical devices are being provided for use in this study as the study intervention is supplied with a Philips InnoSpire Go hand-held vibrating mesh nebulizer. To fulfill regulatory reporting obligations worldwide, the Investigator is responsible for the detection and documentation of events meeting the definitions of device deficiency that occur during the study with such devices.

NOTE: Device deficiencies fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3 and Appendix 4 of the protocol.

8.3.8.1 Time Period for Detecting Medical Device Deficiencies

- Medical device deficiencies that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

- If the Investigator learns of any device deficiency at any time after a participant has been discharged from the study, and such deficiency is considered reasonably related to a medical device provided for the study, the Investigator will promptly notify the Sponsor.

The method of documenting medical device deficiency is provided in Appendix 5.

8.3.8.2 Follow-up of Medical Device Deficiencies

- Follow-up applies to all participants, including those who discontinue study intervention.
- The Investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.
- New or updated information will be recorded on the originally completed form with all changes signed and dated by the Investigator.

8.3.8.3 Prompt Reporting of Medical Device Deficiencies to Sponsor

- Medical device deficiencies will be reported to the Sponsor within 24 hours after the Investigator determines that the event meets the protocol definition of a medical device deficiency.
- The method of reporting medical device deficiencies is provided in Appendix 5.
- The Sponsor will be the contact for the receipt of device deficiency reports.

8.3.8.4 Regulatory Reporting Requirements for Medical Device Deficiencies

- The Investigator will promptly report all device deficiencies occurring with any medical device provided for use in the study in order for the Sponsor to fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.
- The Investigator, or responsible person according to local requirements (e.g., the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of deficiencies to the IRB/IEC.

8.4 Treatment of Overdose (Not Applicable)

There is no risk of overdose.

8.5 Pharmacokinetics (Not Applicable)

Pharmacokinetic parameters are not evaluated in this study.

8.6 Pharmacodynamics (Not Applicable)

Pharmacodynamic parameters are not evaluated in this study.

8.7 Genetics (Not Applicable)

Pharmacogenomics are not evaluated in this study.

8.8 Biomarkers (Not Applicable)

Biomarkers are not evaluated in this study.

8.9 Medical Resource Utilization and Health Economics (Not Applicable)

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 Statistical Considerations

9.1 Statistical Hypothesis

RESP301 reduces the rate of progression to level 5 and above in the modified WHO ordinal scale in COVID-19 (see Section 8.1.1).

This represents a composite endpoint of (i) death (level 7), (ii) hospitalized, on invasive mechanical ventilation or ECMO (level 6), or (iii) hospitalized, on non-invasive ventilation or high-flow oxygen devices (level 5).

9.2 Sample Size Determination

A total of 300 hospitalized participants with confirmed COVID-19 will be randomized 2:1 to receive RESP301+SOC (200 participants) or SOC alone (100 participants).

The comparison between the treatment arms for the primary endpoint will have approximately 80% power and alpha level 0.025 (one-sided) to demonstrate significant reductions (15% versus 30%) of the primary endpoint between RESP001+SOC and the Control arm (SOC), a 50% relative reduction for the proportion of participants who progress to level >4 (see definition of the primary endpoint).

Two interim analyses are planned (see Section 9.5 for details):

1. The first IDMC interim analysis will take place after the first 60 participants have completed Day 7 of the study to evaluate whether the study can be stopped for futility based on change from baseline in room air SpO_2.
2. The second interim analysis will take place after 150 participants have completed Day 14 post-randomization based on event rate for the primary endpoint. The purpose of the second interim analysis will be futility as well as a potential sample size re-estimation in case the actual results differ from the original assumptions.

In addition to the review of efficacy data, safety will be assessed at each of the interim analysis by the IDMC. Further details are provided in Appendix 6.

9.3 Populations for Analyses

For purposes of analysis, the following analysis sets are defined:

Table 9-1 Populations for Analysis

Population (Analysis Set)	Description
Intent-To-Treat (ITT) Population	The ITT Population will include all randomized participants. The ITT participants will be analyzed according to randomized treatment, irrespective of the actual treatment received. Participants who withdraw from treatment early will be followed for the assessment of the Day 14 primary endpoint. All efficacy analyses will be performed using the ITT Population.
Per Protocol (PP) Population	The PP Population will include all participants in the ITT Population with no major protocol deviations that may significantly impact data integrity or patient safety. The PP Population will be used for supportive analyses of the efficacy measurements.
Safety Population (SP)	The SP will include all randomized participants who inhale any amount of study intervention or are randomized to the control arm. The SP will be analyzed according to the actual treatment received. This set will be used for the safety analyses.

The ITT Population will be the primary analysis set for all efficacy analyses and the PP Population will be used to demonstrate robustness of results for the primary efficacy endpoint.

9.4 Statistical Analyses

Below is a description of planned statistical analyses. Further details are presented in the Statistical Analysis Plan (SAP).

9.4.1 General Considerations

All statistical analyses will be conducted using SAS, Version 9.4 or later. Baseline characteristics will be summarized by treatment arm. For continuous measures the mean and standard deviation (SD) will be summarized. Categorical variables will be described by the proportion in each category. In addition, 95% confidence intervals (CIs) will be computed as indicated.

All the categorical variables including the primary endpoint will be summarized by treatment with the numbers and percentages of the participants. Treatment difference will be tested using a Cochran–Mantel–Haenszel test stratified by 1) country and 2) participant's clinical status at Baseline. For the categorical endpoints, relative risk and its 95% CI will be presented.

All of the continuous variables, including the changes from Baseline, will be summarized by treatment with the means, SD, medians and the ranges. The mixed model with repeated measurements /analysis of covariance model with treatment, country, participant's clinical status at Baseline and visit as the model term, and Baseline value as the covariate will be used to test for the significance of the treatment difference. Least square means, standard errors, 95% CIs and p-values will be presented.

Time to event endpoints will be analyzed using the Cox Proportional Hazard model with treatment, participant's clinical status at Baseline and country as the model term. The hazard ratio of RESP301+SOC versus SOC will be presented along with 95% CI and p-value from the model. The Kaplan-Meier curves of the time to events will be presented by treatment for each applicable endpoint.

Handling of missing data

If participants are discharged from the hospital prior to Day 14 due to improvement of the clinical status and their status on Day 14 cannot be obtained, their status on Day 14 for the primary endpoint will be imputed with the status on the day of discharge. Depending on the reasons for missing data on the primary endpoint up to Day 14, additional sensitivity analyses will be performed. Further details on handling on missing data will be provided in the SAP.

9.4.2 Primary Endpoint

The primary endpoint is the proportion of participants who progress to level >4 of the modified WHO ordinal scale due to COVID-19 by Day 14.

9.4.3 Secondary Endpoint(s)

The key secondary endpoints are:

1. Change from baseline on the modified WHO ordinal scale at each visit up to Day 28
2. Change in room air SpO_2 from baseline over time
3. Change in NEWS 2 symptom score from baseline over time
4. Time to improvement to a lower level (<4) of the modified WHO ordinal scale
5. Time to progression to a higher level (>4) of the modified WHO ordinal scale

Additional secondary endpoints are:

1. Time to hospital discharge
2. Incidence of mortality by Day 28

9.4.4 Tertiary/Exploratory Endpoint(s)

CCI

- CCI

9.4.5 Other Safety Analyses

All safety analyses will be performed on the Safety Population.

1. Safety and tolerability assessed by clinical safety laboratory measurements, physical examinations, vital signs, concomitant medications; cumulative incidence of AEs, SAEs and severe AEs
2. Incidence of participants unable to tolerate nebulization due to:
    - Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
    - Other clinical signs of intolerance according to Investigator's opinion
3. Incidence of clinical bronchial hyper-responsiveness related to nebulization

9.4.5.1 Adverse Events

Adverse Events will be coded using the MedDRA coding dictionary.

The number and percentage of patients with any AE, any related AE, any SAE, any related SAE, any severe AE, and related severe AE as well as the total number of events for each category will be summarized. The number of deaths due to an AE, hospitalization due to an AE, and study discontinuation due to an AE will be summarized.

The number and percentage of patients with an AE, as well as the total number of AEs, will be summarized by SOC and preferred term. This tabulation will be repeated for related AEs, SAEs, related SAEs, severe AEs, and related severe AEs.

All AEs will be provided in patient listings. Patient listings of AEs causing discontinuation of study medication, AEs leading to death, SAEs, related AEs, and severe AEs will be produced.

9.4.5.2 Clinical Laboratory Evaluation

Baseline is defined as the last non-missing value obtained at the screening visit and prior to the first exposure to study drug. Actual values and changes from Baseline clinical laboratory tests will be summarized by study day. If more than one laboratory result is reported per study visit per parameter, the last non-missing result will be selected for change from Baseline analysis.

Laboratory test results will be classified according to the reference ranges and clinical significance as determined by the Investigator. The number of patients with a non-missing result, the number and percentage of patients with a clinically significant result less than the lower limit of normal, non-clinically significant result more than the ULN, and clinically significant result more than the ULN will be summarized by study visit. If more than one laboratory result is reported per study day per parameter, the result yielding the most severe classification will be selected for analysis.

Categorical laboratory test results (urinalysis excluding pH) will be summarized by study visit. If more than one laboratory result is reported per study day per parameters, the result yielding the most severe classification will be selected for analysis.

Patients with clinically significant abnormal laboratory test results will be listed. This listing will include all results of the laboratory parameter that was abnormal and determined to be clinically significant by the Investigator for a patient across study visit.

9.4.5.3 Vital Signs

Baseline is defined as the last non-missing value obtained in screening and prior to the first exposure to study drug. Actual values and changes from Baseline in vital signs will be

summarized by study day and study time point. All vital sign data will be presented in patient listings.

Vital sign values will be classified according to the clinical significance as determined by the Investigator. The number of patients with a non-missing result, the number and percentage of patients with a non-clinically significant result, and clinically significant result will be summarized by study visit and study time point. If more than one vital sign result is reported per study day and study time point per parameter, the result yielding the most severe classification will be selected for analysis.

Patients with clinically significant vital sign values will be listed. This listing will include all results of the vital sign parameter that was determined by the Investigator to be clinically significant for a patient across study time points.

9.4.5.4 Physical Examination

Abnormal physical examination findings will be listed.

9.4.6 Other Analyses

Other analyses may be added to the SAP as applicable.

9.5 Interim Analyses

Two interim analyses will be performed.

1. The first analysis will be conducted when about 60 participants (40 participants in the RESP301+SOC arm and 20 participants in the SOC arm) have completed the Day 7 assessment. The purpose of this analysis is to evaluate efficacy of RESP301 with the application of a futility criterion based on the results on SpO_2 change from baseline on Day 7. The following futility criterion will be used for this first interim analysis:

   If the difference in the percentage of participants with at least a 2% improvement in SpO2 between both treatment arms is less than 5%, benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

   As no other modification of the study at the time of the first interim analysis is considered, no adjustment of the alpha level is required.

2. The second interim analysis will be conducted after 150 participants (100 participants in the RESP301+SOC arm and 50 participants in the SOC arm) have completed the Day 14 assessment. The purpose of this analysis is the assessment of futility or a sample size re-estimation (increase only) in case the actual results differ from the original assumptions for the power calculations of the study, related to the percentage of participants meeting the primary endpoint in the control arm and/or the relative treatment benefit achieved in the RESP301+SOC arm compared to the SOC arm.

   To account for the multiple testing due to the second interim analysis an adjustment for the type I error alpha will be applied using the Haybittle-Peto approach which would spend one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis. The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration will be given to stop the study for futility. At the same time sample size re-estimation will be performed to achieve 80% power at the end of the study. The methodology for the adjustment and the procedure to maintain the type I error level will be described in greater detail in the SAP.

Detailed information, including the boundaries futility and characteristics for the sample size re-estimation at the time of the interim analyses will be provided in the SAP and Data Monitoring Committee (DMC) Charter. Further details are also provided in Appendix 6.

9.6 Data Monitoring Committee (DMC)

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet to review after 10 and 20 participants have completed as well as at the time of the interim analyses (ie, after 60 and 150 participants have completed; see Section 9.5).

The detailed procedures and criteria of the interim analyses will be described in the DMC Charter.

10 Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
    - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
    - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The Investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC.
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures.
    - Overall conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH GCP guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Financial Disclosure

Information on financial disclosure can be found in the study monitoring plan.

10.1.3 Informed Consent Process

The Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IEC/IRB and signed by all participants subsequently enrolled in the study as well as those currently enrolled in the study.

10.1.4 Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committees Structure

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study (see Sections 9.5 and 9.6).

10.1.6 Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategies (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for at least 2 years after the last approval of a marketing application or 15 years from completion of the study, whichever is longer according to the relevant local laws and/or regulations. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

- All data generated by the site personnel will be captured electronically at each study center using eCRFs. Data from external sources (such as laboratory data) will be imported into the database. Once the eCRF clinical data have been submitted to the central server at the independent data center, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.
- If additional corrections are needed, the responsible monitor or data manager will raise a query in the electronic data capture (EDC) application. The appropriate staff at the study site will answer queries sent to the Investigator. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will freeze the eCRF page.
- The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the EDC system/eCRF.

10.1.7 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

10.1.8 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the first participant signing the informed consent form and will be the study start date.

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the Investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up

10.1.9 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.10 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first participant is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/Competent Authority approval prior to implementation (if appropriate). In the US: Following approval, the protocol

amendment(s) will be submitted to the Investigational New Drug application under which the study is being conducted.

Administrative changes (not affecting the participant benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.1.11 Liability and Insurance

10.1.11.1 Access to Source Data

Access to source data is described in the Monitoring Plan.

10.2 Appendix 2: The NEWS 2 Scoring System

Chart 1: The NEWS 2 scoring system

Physiological parameter	Score						
	3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

Abbreviations: CVPU: C=new onset confusion, disorientation or agitation, V=responds to voice, P=responds to pain, U=unresponsive; SpO_2=oxygen saturation.

Source: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2

10.3 Appendix 3: Clinical Laboratory Tests

The tests detailed in Table 1-1 will be performed by the local laboratory.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol. Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 10-1 Protocol-Required Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	CBC without differential: White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet, mean platelet volume Methemoglobin			
Clinical Chemistry[1]	Blood urea nitrogen Creatinine Glucose non-fasting	Potassium Sodium Chloride Bicarbonate/CO_2	Aspartate Aminotransferase / Serum Glutamic-Oxaloacetic Transaminase Alanine Aminotransferase/ Serum Glutamic-Pyruvic Transaminase Alkaline phosphatase / Lactate dehydrogenase	Total and direct bilirubin Coagulation (PT/INR, aPTT)
Other Screening Tests	Serum TB test for suspected or confirmed untreated, active tuberculosis.			

Laboratory Assessments	Parameters
	The results of each test must be entered into the eCRF.
NOTES: 1 Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1. All events of ALT ≥3 × ULN and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).	

Investigators must document their review of each laboratory safety report.

10.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1 Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (e.g., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.4.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization (Applies only during the safety follow up period for patients who may have been discharged during the treatment period, ie, between Day 1 and Day 10)

- In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or intervention that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are considered AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective intervention of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive intervention in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.4.3 Recording and Follow-up of AE and SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event. • The Investigator will then record all relevant AE/SAE information in the CRF. • It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to the clinical research organization (CRO) in lieu of completion of the AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by the CRO. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to DMC members or for Medical Monitor review. • The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories: • Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities. • Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe. An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report in the electronic CRF/EDC. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to electronic CRF/EDC.**
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the DMC to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed CRF.

- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.4.4 Reporting of SAE

SAE Reporting to DRC via Electronic Data Collection Tool

The Investigator must report any SAEs to the Parexel Safety Services within 24 hours of becoming aware of the event.

All SAEs will be recorded from signing of informed consent until the end of the study. Serious adverse events occurring after the end of the study and coming to the attention of the Investigator must be reported only if they are considered (in the opinion of the Investigator) causally related to study intervention.

- The primary mechanism for reporting an SAE to DMC will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- The Investigator and the Sponsor (or Sponsor's designated agent) will review each SAE report and the Sponsor/Parexel will evaluate the seriousness and the causal relationship of the event to study intervention. In addition, the Sponsor (or Sponsor's designated agent) will evaluate the expectedness according to the reference document (Investigator Brochure or Summary of Product Characteristics). Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by email.
- Contacts for SAE reporting can be found in the Investigator Manual.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any AE that is a SUSAR has additional reporting requirements, as described below.

- If the SUSAR is fatal or life-threatening, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within seven calendar days after the Sponsor learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information should be reported within an additional 8 days (15 days total).
- If the SUSAR is not fatal or life-threatening but is otherwise serious, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within 15 calendar days after the Sponsor learns of the event.

The Sponsor will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of participants. Follow-up information may be submitted if necessary.

The Sponsor will also provide annual safety updates to the regulatory authorities and IECs responsible for the study. These updates will include information on SUSARs and other relevant safety findings.

All SAEs that occur during the study, and all SAEs occurring up to 18 days after receiving the last dose of study intervention, whether considered to be associated with the study intervention or not, must be reported within 24 hours by telephone or fax to the Parexel Safety Contact using the numbers in the List of Study Personnel.

The minimum information required for an initial report is:

Name of person sending the report (e.g., name, address of Investigator);

- Participant identification (screening/randomization number, initials, NOT participant name);
- Protocol number;
- Description of SAE;
- Causality assessment, if possible.

However, as far as possible all points on the SAE form should be covered in the initial report, or the completed SAE form itself must be emailed or faxed to the Parexel Safety Services. In addition, the event must be documented in the electronic CRF/EDC system.

After receipt of the initial report, the safety center will review the information and, if necessary, contact the Investigator, to obtain further information for assessment of the event. Parexel will be responsible for all information processing and reporting according to local legal requirements. Where necessary, Investigators will inform regulatory authorities in their own countries.

10.5 Appendix 5: Medical Device Adverse Events (AEs) Adverse Device Effects (ADEs), Serious Adverse Events (SAEs) and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-up, and Reporting

The definitions and procedures detailed in this appendix are in accordance with ISO 14155.

Both the Investigator and the Sponsor will comply with all local medical device reporting requirements.

See Section 6.1.1 for the list of medical devices for this study.

10.5.1 Definition of AE and ADE

AE and ADE Definition
An AE is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory finding) in study participants, users, or other persons, whether or not related to the investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices. An ADE is defined as an AE related to the use of an investigational medical device. This definition includes any AEs resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.5.2 Definition of SAE, SADE and USADE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is an AE that:
a. Led to death

b. Led to serious deterioration in the health of the participant, that either resulted in: A life-threatening illness or injury. The term 'life-threatening' in the definition of serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe A permanent impairment of a body structure or a body function, Inpatient or prolonged hospitalization planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
c. Led to fetal distress, fetal death or a congenital abnormality or birth defect
SADE definition
A SADE is defined as an ADEs that has resulted in any of the consequences characteristic of a SAE.
USADE definition
A USADE is a serious ADEs which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

10.5.3 Definition of Device Deficiency

Device Deficiency definition
A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

10.5.4 Recording and Follow-Up of AE and/or SAE and Device Deficiencies

Device deficiencies fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3 and Appendix 4 of the protocol.

10.6 Appendix 6: Statistical Design Considerations

10.6.1 Some General Design Considerations

1. Binary primary endpoint: progression to critical stage/death up to Day 14
    - to be analyzed by a Cochran-Mantel-Haenszel (CMH) stratified for the factors used in the stratified randomization
    - subjects who withdraw from study treatment will be followed up until their primary endpoint outcome is known (i.e., up to Day 14 or progression to critical stage/death, whatever is first)
    - it is, therefore, not clear what current protocol synopsis text could mean: *If subjects are discharged from the hospital prior to day 14 due to improvement of the clinical status and their status on day 14 cannot be obtained, their status on day 14 for the primary endpoint will be imputed with the status on the day of discharge. and what the implications on statistical study characteristics could be. Further discussion is needed.*
2. Timing of the two planned interim analyses with stopping options is stated as

There are 2 interim analyses planned, the first interim analysis after the first 60 patients have completed day 7 of the observation period … and a second interim analysis after 150 patients have completed day 14 of the observation period.

With the current text for the timing of the first interim analysis, it is not clear how many subjects will have been randomized at least 14 days prior to the interim data cut-off date and can therefore provide data for the binary primary endpoint ("progression to critical stage/death up to Day 14").

For such a binary endpoint, Parexel does not recommend including any subject randomized less than 14 days prior to the interim data cut-off date (note this would be different to a study with a time-to-event endpoint) as

- for such subjects not yet progressed, the outcome up to Day 14 is unknown and should not be imputed as "not progressed up to Day 14"
- for such subjects progressed prior to Day 14, the outcome is known but their inclusion would bias the estimation of progression probability up to Day 14 in a upwards direction.

In order to set up the adaptive group-sequential study design, the following assumptions have been used:

- first interim analysis: a total of ≈ 39 subjects randomized at least 14 days prior to data cut-off date
- second interim analysis: a total of ≈ 50% of the initially planned number of subjects randomized at least 14 days prior to data cut-off date.

3. Non-binding DMC guidance for stopping the study for futility at the first interim analysis is stated as:

If the difference in the percentage of patients with at least a 2% improvement in SpO2 between both treatment arms is less than 5%, a benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

As the main futility stopping criterion is based on improvement in SpO2 (and not on the primary efficacy endpoint), this first interim analysis will be "ignored" in these statistical considerations for an adaptive group-sequential design. We may revisit the first interim analysis at a later point in time when more information is available.

4. Non-binding DMC guidance for stopping the study for futility at the second interim analysis stated as:

The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the trial will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration will be given to stop the study for futility.

5. Current DMC guidance for stopping the study for superior efficacy at the second interim analysis is stated as:

To account for the multiple testing due to the second interim analysis an adjustment for the type-I-error probability will be applied using the Haybittle-Peto approach with one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis.

6. For initial sample size calculation, the following additional assumptions / features from the protocol have been used

- progression probability up to Day 14 for Standard of Care (SOC): 30%

- progression probability up to Day 14 for RESP301+SOC: 15%
- desired power assuming the alternative hypothesis stated above: 80%
- 2:1 randomization

7. Method for adaptation of the sample size based on the second interim analysis data is stated as

A sample size re-estimation will be performed to achieve 80% conditional power at the end of the study. The methodology for the adjustment and the procedure to maintain the type-I-error level will be described in greater detail in an appendix to the protocol. The maximum increase of the sample size will be limited to a total number of 600 randomized subjects.

Parexel has put a suggestions with some fictitious interim data for illustration in Section 10.6.3; that section also illustrates by simulations the Cui/Hung/Wang (CWH) approach using a weighted (weights fixed at the design-stage) test for the final analysis versus the Chen/DeMets/Lan (CDL) approach without such weights at the final analysis.

10.6.2 General Design Characteristics

Parexel proposes to base non-binding futility stopping criteria on conditional power criteria. Conditional power (CP) is the conditional probability for achieving statistically significant superiority of RESP301+SOC over SOC at the final analysis, given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study (an alternative would be predictive power (PP), a Bayesian version of CP where prior distributions and observed interim results are combined to obtain the probability for achieving statistically significant superiority of gimsilumab over placebo at the final analysis).

Randomization 2:1	**Group-Sequential Design**
1st Interim Analysis (IA) 2nd IA	N ≈ 39 for D14 ≈ 50% for D14
Futility criterion 1st IA Futility criterion 2nd IA Superior efficacy criterion 2nd IA	Based on SpO_2 CP < 4% p < 0.0005
Total sample size required for 80% power	**300**
Futility stopping probability at 2nd IA under "No effect" Futility stopping probability at 2nd IA under "Planned effect 30% vs 15%"	1st IA: **NA** 2nd IA: **70%** 1st IA: **NA** 2nd IA: **6%**
Superiority stopping probability at 2nd IA under "Planned effect 30% vs 15%" Superiority stopping probability at 2nd IA under "30% vs 10%"	**10%** **28%**

10.6.3 Adaptive Sample Size Re-Estimation

Another objective of the 2nd interim analysis is to re-estimate the sample size based on the unblinded interim results.

Proposed procedure at 2nd interim analysis (planned to include approximately 150 subjects)

- unblinded analysis of the primary efficacy endpoint ("progression to level >4 of modified WHO ordinal COVID-19 scale by Day 14" as a binary endpoint)
- calculation of conditional power (CP) given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study
    - if CP < 4%: recommend early stop for futility
    - if one-sided p-value < 0.0005: recommend early stop for superior efficacy
    - if 50% < CP < 80% and one-sided p-value ≥ 0.0005:
        - continue the study with an increased total sample size N*, so that CP with a total of N* subjects is increased to 80%, same value as the (unconditional) desired power in the study design
        - N*, however, is limited to 600 (which is twice the original sample size 300)
    - if 4% ≤ CP ≤ 50%: continue the study without a change in sample size (otherwise, the sample size would need to be increased too much or the maximum sample size of 600 would not be sufficient to come close to a CP of 80%.

The table on the next page illustrated the proposed procedure for a number of possible results observed at the 2nd interim analysis.

Table with examples for the adaptive sample size procedure described above using the Cui/Hung/Wang (CHW) approach using a weighted (fixed weights determined by the stage-wise sample sizes planned at the design-stage) test for the final analysis:

2nd IA results with n=150 subjects (50 for SOC, 100 for RESP301+SOC		**CP under observed trend for planned 300**	**Total sample size required for CP 80% under observed trend**	**CP under observed trend if total sample size capped by 600**
15 (30%)	5 (5%)	$p < 0.0005$ → early stop for superior efficacy		
15 (30%)	10 (10%)	99%	Not applicable	Not applicable
15 (30%)	15 (15%)	90%	Not applicable	Not applicable
15 (30%)	18 (18%)	66%	393	Not applicable
15 (30%)	19 (19%)	55%	483	Not applicable
15 (30%)	21 (21%)	34%	[798]	[67%]
15 (30%)	26 (26%)	3.99%	Early stop for futility	
12 (24%)	10 (10%)	92%	Not applicable	Not applicable
12 (24%)	12 (12%)	77%	321	Not applicable
12 (24%)	14 (14%)	54%	498	Not applicable
12 (24%)	15 (15%)	42%	[646]	[77%]
12 (24%)	18 (18%)	14%	[1800]	[31%]
12 (24%)	20 (20%)	2.6%	Early stop for futility	
10 (20%)	8 (8%)	85%	Not applicable	Not applicable
10 (20%)	10 (10%)	64%	412	Not applicable
10 (20%)	11 (11%)	50.4%	534	Not applicable

10 (20%)	13 (13%)	26%	[1014]	[55%]
10 (20%)	15 (15%)	10%	[2460]	[23%]
10 (20%)	18 (18%)	1.4%	Early stop for futility	

Quantities in [ ] indicate theoretical values as sample size would not be increased per the currently proposed adaptive design.

10.6.4 Overall Adaptive Design Performance

Finally, the Cui/Hung/Wang (CHW) approach is compared to the Chen/DeMets/Lan (CDL) approach without pre-defined fixed weights for the final analysis (which is valid as sample size may only be increased if CP (obtained under observed trend) at the 2nd interim analysis exceeds 50%).

All results below are for the group-sequential design with adaptive sample size re-estimation procedure as described in previous sections and based on 10.000 simulated trials each.

Scenario 1: true progression probabilities are 30% (SOC) and 15% (RESP301+SOC)


CHW Simulation 0.3 vs 0.15.html


CDL Simulation 0.3 vs 0.15.html

Scenario 2: true progression probabilities are 24% (SOC) and 14% (RESP301+SOC)


CHW Simulation 0.24 vs 0.14.html


CDL Simulation 0.24 vs 0.14.html

Scenario 3: true progression probabilities are 30% (SOC) and 10% (RESP301+SOC)


CHW Simulation 0.3 vs 0.1.html


CDL Simulation 0.3 vs 0.1.html

Scenario 4: true progression probabilities are 24% (SOC) and 20% (RESP301+SOC)


CHW Simulation 0.24 vs 0.2.html


CDL Simulation 0.24 vs 0.2.html

10.7 Appendix 7: Abbreviations

ADE	Adverse device effect
AE	adverse event
ALT	alanine aminotransferase
ASADE	Anticipated serious adverse device effect
AST	aspartate aminotransferase
CBC	complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CP	Conditional power
CRF	case report form(s) (paper or electronic as appropriate for this study)
CRO	contract research organization
CV	cardiovascular
DMC	Data Monitoring Committee
DRE	disease-related events
ECG	electrocardiogram
ECMO	Extra corporeal membrane oxygenation
EDC	electronic data capture
EoS	End of study
FDA	Food and Drug Administration
FiO_2	fraction of inspired oxygen
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board

ITT	Intent-to-treat
IVRS	interactive voice response system
IWRS	interactive web response system
LFT	Liver function test
mHb	Methemoglobin
NEWS	National Early Warning Score
NHS	National Health Service
NO	Nitric oxide
NO_2	Nitrogen dioxide
NOx	Oxide of nitrogen
RT-PCR	Reverse transcriptase polymerase chain reaction
PP	Per protocol
PT	Prothrombin time
QTcF	QT interval corrected using Fridericia's formula
SADE	Serious adverse device effect
SAE	serious adverse event
SAP	statistical analysis plan
SD	Standard deviation
SoA	schedule of activities
SOC	Standard of care
SP	Safety population
SpO_2	Oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
TB	Tuberculosis
TID	Three times daily
ULN	Upper limit of normal
USADE	Unanticipated serious adverse device effect
WHO	World Health Organization

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Investigator Agreement Page

Declaration of the Principal or Global Coordinating Investigator

Title: NOCoV2 - An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the *Declaration of Helsinki* and the guidelines on Good Clinical Practice.

Principal or Global Coordinating Investigator

PPD

Title Page

Protocol Title:	**An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen (NOCoV2)**
Short Title:	An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301+SOC vs SOC in hospitalized participants with COVID-19 requiring supplemental oxygen
Compound:	RESP301
Indication:	Mild to moderate COVID-19
Study Sponsor:	Thirty Respiratory Limited PPD London, W1K 6PL, United Kingdom
Protocol Number.:	RESP301-002
Study Phase:	Phase 2/Phase 3
Regulatory Agency Identifying Number:	IND No: Pending EudraCT No: 2020-002120-37
Approval Date of Current Version:	Final, 05 Jun 2020; Amendment 1 (Version 2.0)
Date and Version of Previous Protocol:	01 May 2020; Version: 1.0

Sponsor Signatory:

PPD

5th June 2020

PPD

Date

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Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY		
Document	**Version**	**Date**
Amendment 1.0	Version 2.0	05-Jun-2020
Original Protocol	Version 1.0	01-May-2020

Amendment 1.0 (05-Jun-2020)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the EU.

Overall Rationale for the Amendment:

The original protocol was updated to include important safety guidelines for study intervention administration for the first 10 participants (ie, up to the first independent data monitoring committee [IDMC] safety review). It was also updated to align with the IB Version 2.0, dated 27 May 2020. Other changes, with brief rationale, are summarized in the following table.

Section # and Name	Description of Change	Brief Rationale
Global change	Device name Philips InnoSpire Go was replaced with generic reference to a vibrating mesh nebulizer.	The decision was made to keep the device generic for flexibility.
Section 1.1 Synopsis and Section 4.1 Overall Design	Clarified that participants were (male or female) adults. Wording of study design also clarified to include study intervention and minor clarification to text for screening period.	Updated to align with the IB Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis, Section 4.1 Overall Design, Section 7.1, Discontinuation of Study Intervention	Bullets specifying reasons for permanent discontinuation updated to reflect content of Section 7.1.	Correction, for consistency across the protocol.
Section 1.2 Schema	Dose of study intervention corrected to 6.0 mL. Minor clarifications added in footnotes.	Correction.
Section 1.2 Schema	Footnote 2 updated.	Text added to make it clear that participants will be allowed sufficient time to consider their participation in the study.
Section 1.1 Synopsis, Section 1.3 Schedule of Activities (SoA), Section 2.2.1 Risk Assessment, Section 4.1 Overall Design, and Section 6.1 Study Intervention Administered	The following statement was added: A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). RESP301 will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6). The time gap between consecutive doses in the 3 times per day (TID) schedule was updated to “at least 6 hours” from “at least 4 hours”.	Updated to align with the Investigator Brochure (IB) Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 SoA, Section 5.2 Inclusion Criteria, Section 8.3.5 Pregnancy, Appendix 3, Clinical Laboratory Tests	Addition of urine pregnancy test at screening. Requirement for all females of childbearing potential, including pregnant females, to consent to urine pregnancy testing at screening. Details of pregnancy follow-up procedures added.	All women, including women of childbearing potential and pregnant women, are allowed in the study. Therefore, it is necessary to determine pregnancy status at study entry so that pregnant females can be followed to determine the outcome of the pregnancy.
Section 1.3 SoA, Appendix 3, Clinical Laboratory Tests	Deletion of tuberculosis (TB) screening test. Nitrite added to the protocol-required laboratory assessments (End of Study [EoS] only).	Screening TB test is not mandatory for this study. Nitrite is to be measured for participants who are still hospitalized at EoS.
Section 1.3 SoA and Section 7.1 Permanent discontinuation of Study Intervention	Deletion of electrocardiogram (ECG) testing after screening. Clarified that significant changes in ECGs would be identified only as part of normal clinical follow-up.	Only routine ECG monitoring is needed for this study.
Section 2.1 Background, Section 11 References	Text deleted from end of paragraph 12. References no longer cited were removed from Section 11.	Removal of text that was specific to the Philips InnoSpire Go nebulizer.
Table 2-1, Risk Assessment and Section 11 References	The risk assessment table was updated to align with the above changes and IB. A new reference (Brannan et al, 2005) was added to Section 11 to support additional text around risk of bronchospasm.	Updated to align with the IB Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis, Section 1.3 SoA, Section 4.1, Overall Design	Visit window for Day 14 and Day 28 follow-up extended to (± 2 days).	To allow more flexibility.
Section 1.3 SoA	Footnote 2 corrected to include weight and exclude ECG. Clarified that laboratory testing at EoS follow up is for nitrite and methemoglobin only.	Correction and clarification.
Section 1.3 SoA	Footnote 6e corrected from 'Recording of SpO_2 * pre-nebulization on ambient air' to 'Recording of SpO_2 * on ambient air'.	'pre-nebulization' was removed as SpO_2 measurement on ambient air is required for both active and control group.
Section 1.3 SoA	Footnote 7 amended to clarify text that is applicable to participants in the active arm only.	Clarification.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 SoA, Section 8.3 Adverse Events and Serious Adverse Events, Section 8.3.8 Medical Device Deficiencies, and Appendix 5: Medical Device Adverse Events (AEs) Adverse Device Effects (ADEs), Serious Adverse Events (SAEs) and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-up, and Reporting	Changes related to the removal of Appendix 5. Table 8-1 deleted.	Appendix and table were not required because the nebulizer device used in the study is licensed and there is no requirement to collect data on the device.
Section 2.1 Background	Updated to include a description of the three mechanisms of action of RESP301 in fighting virus infection, including addition of new figure (Figure 2–1).	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 2.2.2 Benefit Assessment	Clarification added regarding choice of nebulizer for this study.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 3 Objectives and Endpoints	Safety endpoint for AEs amended to include both counts and cumulative incidence.	Both types of data will be summarized.

Section # and Name	Description of Change	Brief Rationale
Section 3 Objectives and Endpoints, Section 9.4.4 Tertiary/Exploratory Endpoint(s)	Exploratory endpoint: timepoint for change from baseline measurement of fraction of inspired oxygen (FiO_2) changed from Day 7 to Day 10.	Correction; the endpoint should be measured at end of treatment.
Section 4.4 End of Study Definition	The following clarification was added to the definition: "End of the study is defined as the last participant's last visit or follow up call."	Clarification of definition.
Section 6.1 Study Intervention(s) Administered	This section (including Table 6-1) was updated to include dosing instructions for the first 10 participants (ie, up to the first IDMC safety review). In the event of bronchospasm, rescue bronchodilator can be initiated if needed. A new figure was also added to clearly show the staggered approach (Figure 6–1). The TID dosage was also clarified as every 8 hours with at least 6 hours between consecutive doses. Dosing Instructions were updated to clarify that nasal oxygen may be administered during nebulization if the participant is unable to tolerate nebulization without supplemental oxygen.	Specific study drug administration guidelines (including the use of a rescue medication) were provided to monitor tolerability and response to RESP301 inhalation (as described in the risk-benefit table in Section 2.2.1) and to align with the IB, Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 6.2.1 Preparation of Study Intervention Product	Instructions for mixing the two solutions were updated. Further clarification on the delivered dose was added.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 6.5.1 Rescue Medication	A new section was added to describe allowed bronchodilator rescue medication for participants treated before the first IDMC safety review (short acting beta agonist such as salbutamol/ albuterol according to investigational site standard practice for acute bronchospasm)	Allowed rescue medication was added to minimize the risk of bronchospasm to participants, and to align with the IB Version 2.0, dated 27 May 2020.
Section 7.1 Discontinuation of Study Intervention	Rationale was added for additional monitoring and discontinuation guidance in case of bronchospasm. Table 7-1 added to provide additional clarification on the differences in signs and symptoms for acute bronchospasm and COVID-19 progression.	Safety guidance (including the use of a rescue medication) was updated to minimize the risk of bronchospasm to participants (as described in the risk-benefit table in Section 2.2.1) and to align with the IB Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 7.1 Discontinuation of Study Intervention Section 7.2, Temporary Discontinuation of Study Intervention Subsequent subsections of renumbered to account for new Section 7.2.	Heading updated to "Permanent" Discontinuation of Study Intervention. Text relating to temporary discontinuation moved to new Section 7.2.	Text describing temporary discontinuation now moved into separate section for clarity and consistency.
Section 8.3.1 Time Period and Frequency for Collecting AE and SAE Information	Corrected statement that AEs and SAEs will be collected from signing of informed consent and clarified that SAEs will be reported within 24 hours of the Investigator becoming aware of the SAE, to align with Appendix 4 (previously it stated 'from randomization'). Deleted second paragraph.	Internal document consistency.
Section 8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs	Loss of sense of taste and smell added to the list of common DREs in participants with COVID-19.	Updated in line with updated World Health Guidelines.

Section # and Name	Description of Change	Brief Rationale
Section 9.2 Sample Size Determination, Section 9.5 Interim Analyses, and Appendix 5, Section 10.5.1 Some General Design Considerations	The timing of the interim analysis was changed from when 60 participants have completed the Day 7 assessment to when 60 participants have completed the Day 10 assessment to make it consistent with the planned treatment duration and the SoA.	Internal document consistency.
Section 9.4.5.2 Clinical Laboratory Evaluation	The following sentence was added: Participants who had urine pregnancy test at screening and the results will be listed.	Updated to include pregnancy test data collection.
Appendix 1, Section 10.1.3 Informed Consent Process, and Section 10.1.4 Data Protection	Text added clarifying that a separate optional ICF will be provided for US participants who agree to tokenization.	Added clarification that tokenization is for US participants only.
Appendix 4, Section 10.4.3 Recording of follow-up of AE and SAE, Section 10.4.4 Reporting of SAE	Alignment of reporting details to state 'Parexel Safety Services' throughout appendix. Text was added to include paper SAE data collection tool.	Internal document consistency and inclusion of paper SAE reporting data collection tool.
Appendix 7, Abbreviations	Heading number updated to reflect deletion of Appendix 6. Updated to reflect changes to abbreviations used in the document.	Consistency.

Section # and Name	Description of Change	Brief Rationale
Section 11 References	Five new references were added to support the above updates (Basu et al, Benz et al, Brannen et al, Colosanti et al, and Saura et al). Two references were deleted as they are no longer cited.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Whole document	Minor language and format changes.	For improved clarity and readability.

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1 Protocol Summary

1.1 Synopsis

Protocol Title: An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen (NOCoV2)

Sponsor Protocol No.: RESP301-002

Study Phase: Phase 2/Phase 3

Sponsor: Thirty Respiratory Limited

Rationale:

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design. Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer 3 times a day (TID) for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 4 in the modified World Health Organization (WHO) ordinal scale into levels >4	• Proportion of participants who progress to level >4 of modified WHO ordinal scale due to COVID-19 by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO2	• Change in room air SpO2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time

Objectives	Endpoints
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement to a lower level (<4) of modified WHO ordinal scale • Time to progression to a higher level (>4) of modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	

• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements
	• Physical examinations
	• Vital signs
	• Concomitant medications
	• Counts and cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

Overall Design:

- Open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 added to standard of care (SOC) in hospitalized patients with COVID-19 requiring supplemental oxygen.

- Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as detailed in Section 6.3.

- The study treatment will be permanently discontinued prior to 10 days if the participant:

    - Improves to level 1 or 2 of the modified WHO ordinal scale;
    - Progresses to a level > 4 of the modified WHO ordinal scale;
    - Experiences an event that requires permanent discontinuation as described in Section 7.1.

- An Independent Data Monitoring Committee (IDMC) will be responsible for closely reviewing the safety and efficacy data from interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

- A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). Each RESP301 dose will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6).

Disclosure Statement: This is a parallel group treatment study with two arms that are open-label.

Number of Participants:

Approximately 300 adult (male or female) participants will be randomly assigned to study intervention (200 to the Investigational arm and 100 to the Control arm).

Intervention Groups and Duration:

The total study duration for a participant from screening to last follow up will be up to 30 days (±2 days). The study will be divided into the following periods:

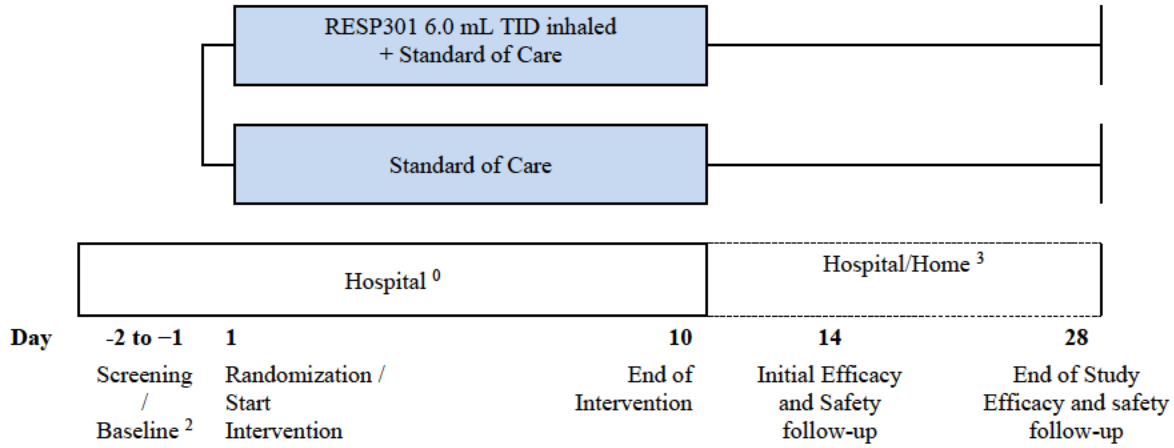
- Screening period: starts up to 2 days (48 hours) prior to and extends up to the day of treatment initiation (Day 1); Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28 (both ±2 days)
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±2 days)

Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer TID for up to 10 days in addition to the SOC, while participants in the Control arm will receive SOC alone.

Data Monitoring Committee: Yes

1.2 Schema

Figure 1–1: Study Design



TID=3 times daily

1 Participants may be discharged from hospital before Day 10.

2 Screening period may include Day 1, as participants may be screened and randomized on the same day provided all eligibility criteria are met and the participant had sufficient time to consider their participation in the study.

3 The post-treatment efficacy and safety follow-ups may be conducted by telephone for participants who are discharged from the hospital at the time.

1.3 Schedule of Activities (SoA)

Table 1-1 Schedule of Activities (SoA)

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Informed consent	X							Optional tokenization ICF to be provided for a subset of participants in the United States (see Section 8.4)
Confirmation of COVID-19 infection by nasopharyngeal RT-PCR [3]	X							
Inclusion and exclusion criteria	X	X						Recheck clinical status before 1st dose of study intervention
Demographics	X							
Physical examination including height and weight	X			X	X		X [2]	Full physical exam and height only at screening

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Pregnancy test (urine)	X							All women, including women of childbearing potential and pregnant women, may be enrolled in the study. Pregnancy status should be recorded (see Section 8.3.5). Note: Only females of child bearing potential and pregnant females need to undergo a screening pregnancy test.
Medical history (includes substance usage)	X							History of substance usage or abuse to be recorded but is not exclusionary
Medication history	X							In the prior 3 months
Laboratory assessments (including liver chemistries) [4]	X	X	X [4]	X	X		X [2]	
12-lead ECG	X							After resting supine for approximately 5 minutes. QRS, QT, QTcF, PR, RR, rate
Vital signs [5]	X	X	X [5]	X	X		X	Within 15 minutes prior to study intervention administration
Randomization		X						

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Study intervention, TID [6]		X	X	X				A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). Each RESP301 dose will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6). Where possible study intervention should be administered at approximately the same times each day At least 6 hours between 2 consecutive doses
AE/SAE review	X	X	X	X	X	X	X	
Concomitant medication review	X	X	X	X	X	X	X	
Oxygen / respiratory assessment [7]	X	X	X	X				
SpO_2 on room air (after at least 1 minute) prior to study intervention administration and immediately at the end of nebulization [6]		X	X	X				See Section 8.1.2
Modified WHO ordinal scale	X	X	X	X		X	X	See Section 8.1.1

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
NEWS 2 assessment [7]		X	X	X				Prior to study intervention in the morning. See Section 8.1.3

Abbreviations: AE=adverse event; aPTT= activated partial thromboplastin time; CBC=complete blood count; Chem 7=blood chemistry panel (sodium, potassium, chloride, bicarbonate/CO_2, blood urea nitrogen, creatinine, glucose); CV=cardiovascular; ECG=electrocardiogram; LFTs=liver function tests; mHb=methemoglobin; PT/INR=prothrombin time/international normalized ratio; RT-PCR=reverse transcription-polymerase chain reaction; SAE=serious adverse event; TID=three times daily.

1. Screening and randomization may be on the same day, providing all eligibility criteria are met.
2. For participants who are already discharged at either the Day 14 or Day 28 (EoS) follow-up, there will be a phone call to check AEs/SAEs, concomitant medications, and modified WHO ordinal scale. At EoS, safety laboratory tests (nitrite and mHb only) and weight will be collected for participants in hospital only.
3. COVID-19 infection must be confirmed and documented in chart with a positive result on a validated test.
4. At Screening: CBC, chem 7, mHb, LFTs, coagulation (PT/INR, aPTT).

   On Day 1, Day 3, Day 7 and Day 10, or early withdrawal: CBC and chem 7 (per SOC).

   On Day 28: nitrite and mHb (participants in hospital at EoS only).
5. Vital signs: temperature, heart rate, blood pressure, respiratory rate. Vital signs to be repeated as per SOC during treatment period.
6. Study intervention comprises the following procedures which are to be done in order and as quickly as possible:
   a. Recording of oxygen flow level including mode (nasal cannula, mask)
   b. Recording of SpO_2 and heart rate
   c. Discontinuation of supplemental oxygen administration *
   d. Close participant oversight by trained health care professional until SpO_2 is stable on ambient air after one minute at least *
   e. Recording of SpO_2 * pre-nebulization on ambient air
   f. Preparation of the admixture of RESP301*
   g. Administration of the nebulization via a vibrating mesh nebulizer while monitoring SpO_2 and heart rate (alarms set to 89% and 120 beats / min, or as indicated by the Investigator, for stopping study intervention administration and resuming supplemental oxygen) * If the participant is unable to tolerate nebulization without supplemental oxygen, nasal oxygen may be administered during nebulization.
   h. Discontinuation of nebulization after 5 minutes or no product left in the device, whichever comes first *
   i. Recording of SpO_2 * post nebulization on ambient air
   j. Resuming supplemental oxygen *
   k. Participant oversight until SpO_2 is stable *
   l. Recording of SpO_2 *

   * *for participants on active treatment arm only*
7. Immediately before participant is discontinued from oxygen for study intervention administration (*for participants on active treatment arm only*) and assessment of SpO_2 at room air.

2 Introduction

2.1 Background

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by a novel coronavirus (SARS-CoV-2). COVID-19 was first described in Wuhan, China, in December 2019 and is now a global pandemic (Matos et al, 2020). Most of those affected have milder illness (80%), 15% will be severely ill (require oxygen) and 5% will require intensive care unit care (Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Of those who are critically ill, most require early intubation and mechanical ventilation. Other complications include septic shock and multi-organ failure, including acute kidney injury and cardiac injury (Yang et al, 2020). Older age and comorbid diseases, such as chronic obstructive pulmonary disease (COPD), hypertension, and diabetes, increase risk of death (Huang et al, 2020; Zhou et al, 2020). The virus is highly contagious and spread via respiratory droplets, direct contact and, if aerosolized, airborne routes. The most common symptoms include fever, fatigue, dry cough, and shortness of breath.

A critical early component of innate host defense in the airway is the ability of respiratory epithelial cells to produce high levels of NO (Kao et al, 2001). NO functions as a signaling molecule in initiation of the inflammatory response to viruses, and also has direct antiviral effects (Folkerts et al, 1998). The airway epithelium has highly efficient nitric oxide (NO) synthetic machinery which is amplified in viral infection. Healthy human airway epithelium has abundant expression of the endothelial enzyme NOS II due to continuous transcriptional activation of the gene in vivo. Loss of NO synthesis in lung diseases predisposes individuals to increased virus/microbe infection (Xu et al, 2006).

The physiological roles of nitric oxide (NO) and the enzymatic pathways for its synthesis via NO synthase have been clearly established for many years (Tucker et al, 2007). In particular, NO has been demonstrated to have potent anti-microbial properties against a wide range of pathogens. An alternative non-enzymatic synthetic pathway for NO synthesis has been developed, which generates NO and related higher oxides of nitrogen (NOx) via the chemical reactions of acidified nitrite (Hardwick et al, 2001, Tucker et al, 1999). A solution of co-mixed NO/NOx and ascorbic acid (RespiNOS), delivered by nebulizer, was found to be safe and well tolerated in healthy volunteers (Tucker et al, 2007). Spectral investigations further confirmed that there were no potentially harmful moieties present in the solution. The study concluded that RespiNOS had potential for use as a broad-spectrum anti-microbial agent in patients with chronic bronchial sepsis such as bronchiectasis, COPD, and cystic fibrosis.

RESP301 is a NO-generating liquid designed to release NO in situ in the upper airways and deep in the alveolar spaces. RESP301 is an admix solution of two precursor solutions mixed together at point of care for immediate inhaled administration via a CE marked handheld nebulizer and has specific advantages (see below) over inhaled NO gas in treating patients with COVID-19 during the current pandemic. It has shown high in vitro activity against respiratory pathogens, both viral and bacterial.

Key advantages of RESP301 over inhaled NO gas are:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

The Sponsor has conducted a number of supportive in vitro studies to assess the activity of NO-generating solutions against major respiratory viruses responsible for infections in humans. These studies include the following:

- In vitro studies to demonstrate the effects of RESP301 on SARS-CoV-2 replication
- In vitro study of RESP301 against influenza A (H1N1) and human rhinovirus
- Determination of the anti-viral efficacy of NO-releasing formulations against two strains of virus (influenza A virus [H1N1] and human rhinovirus 16)
- Anti-microbial activity of acidified nitrite solutions against intracellular drug sensitive and drug resistant *Mycobacterium tuberculosis* and *M. abscessus* infections
- Anti-microbial activity of RESP301 when used alone and in combination with antibiotics

Recent data from experiments at two independent laboratories using two different isolates of SARS-CoV-2 suggest that incubation with RESP301 for 36 to 48 hours reduced viral load to levels below the limit of detection of the assay at concentrations which were not associated with cytotoxicity. The limiting factor of the experiments was the slow growth rate of the viral controls; reduction in viral load was 2.2 and 4 Log_{10} median tissue culture infectious dose ($TCID_{50}$).

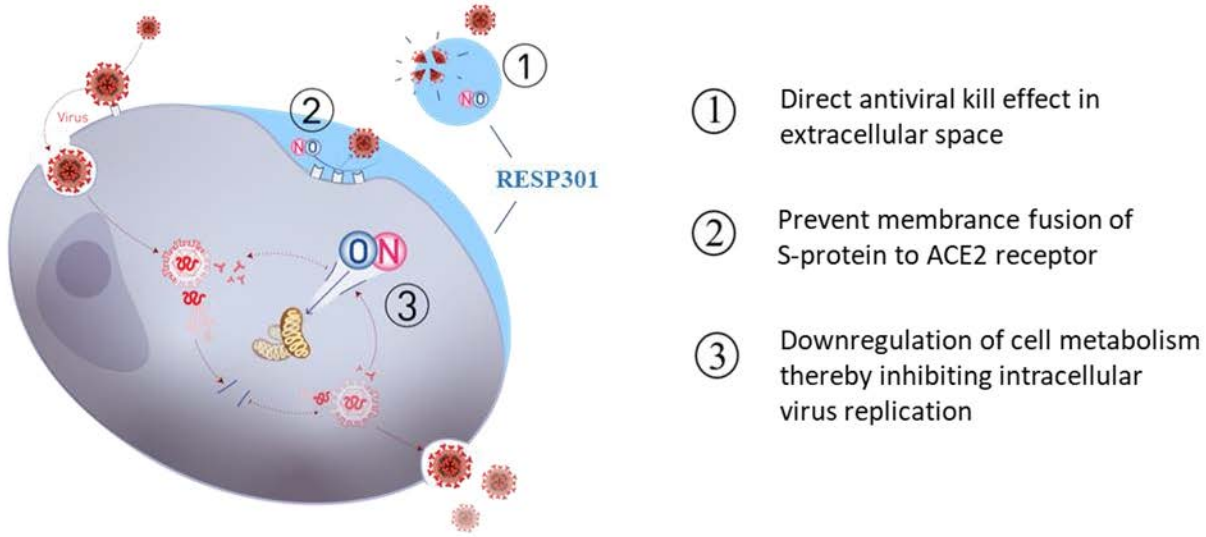
Studies of inhaled nebulized sodium nitrite (AIR001) in healthy subjects and in patients showed that inhaled acidified nitrite produces dose proportional plasma pharmacokinetics without accumulation following repeated administration, with low systemic blood levels of nitrite and

methemoglobin (<3%) (Rix et al, 2015). Inhaled nitrite (AIR001) at doses up to 90 mg three times daily (TID) displayed a good safety profile and is well tolerated (Parsley et al, 2013; Simon et al, 2016), thus supporting the investigation of RESP301 in the clinical setting.

RESP301 delivers NO in a specifically focused way and with a unique mode of administration. It is an important evolutionary step in delivering NO that is more physiological and overcomes the technical difficulties and efficacy limitations of current NO gas therapy. Whereas inhaled NO gas has been used to treat patients with virus-induced acute respiratory distress syndrome and is being tested currently in COVID-19 patients, it focuses on increasing vascular permeability and is not sufficiently targeted at the infection, it is rapidly dispelled with exhalation and is oxidized in air to toxic NO_2. By comparison, RESP301 generates NO in a liquid environment that comes in direct contact with the virus and the virus-infected cells. As a result, RESP301 has a better targeted approach, and lower concentrations of NO are required, with a much more portable delivery system. An important environmental safety factor is that RESP301 produces negligible levels of NO_2 generated and exhaled NO.

In generating NO, RESP301 has at least three distinct mechanisms of action in fighting virus infection, two of which are common to many viruses and one is very specific to coronaviruses, such as SARS-CoV-2 (Figure 2–1).

Figure 2–1: Mode of Action of RESP301 Against SARS-Cov-2



1. Nitric oxide has a direct kill effect on the virus in the extracellular space. Nitric oxide-mediated nitrosylation of viral and host macromolecules appears to block viral replication and this has been demonstrated for several viruses (Saura et al, 1999; Basu et al, 1999). Enzymes, such as proteases (reverse transcriptases, and ribonucleotide reductase), vital for the life-cycle of the virus, are targets for NO nitrosylation (Benz et al, 2002; Colosanti et al, 1999).
2. In coronaviruses like SARS-CoV-2, NO specifically prevents membrane fusion of the S-protein (the spike protein that gives the virus its characteristic crown-like appearance) to the angiotensin-converting enzyme 2 receptor on the host cell.
3. Nitric oxide can attack the virus indirectly. Since viruses are non-living entities and do not have their own metabolism, they hijack the host cell's metabolic processes, and derive their energy and specific cellular substrates from the host cell. Nitric oxide suppresses the virus-induced hyperactivity in the host cell and thereby deprives the virus of its crucial supply chain.

RESP301 efficiently delivers with all three modes of action and is therefore considered an ideal candidate for clinical trials in COVID-19.

A detailed description of the chemistry, pharmacology, efficacy, and safety of RESP301 is provided in the Investigator's Brochure.

2.2 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of RESP301 may be found in the Investigator's Brochure.

2.2.1 Risk Assessment

Table 2-1 Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention RESP301		
Participants may need to be discontinued from supplemental oxygen for	Study treatment is to be administered via a vibrating mesh nebulizer which is designed for oral inhalation.	Participants unable to be safely discontinued from supplemental oxygen will be

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
several minutes to receive nebulized study treatment		excluded (Exclusion criterion 2) Treatment administration is to be performed as quickly as possible (Section 1.3) Oxygen saturation (SpO_2) and heart rate will be monitored throughout the study intervention administration (Section 1.3) In case of decrease in SpO_2 or clinical signs of intolerance to nebulization, RESP301 treatment may be paused temporarily and supplemental O_2 resumed or the study treatment may be discontinued permanently (see Section 7.1) per Investigator assessment.
Risk of methemoglobinemia	Very low levels are produced but as methemoglobinemia has been reported with continuous NO gas administration, the risk cannot be firmly ruled out	Participants with a history of methemoglobinemia will be excluded (Exclusion criterion 4) and methemoglobin (mHb) is included in safety laboratory tests (Section 1.3)
Risk of clinical bronchial hyper-responsiveness related to nebulization	Excipients in the nebulized RESP301 include mannitol which is a known bronchial	Participants with a known history of moderate or severe bronchial hyperreactivity

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	irritant at the average dose of a positive mannitol test (239.0 ± 185.0 mg, Brannan et al, 2005). While the dose used in this study (54.66 mg) is lower than the average dose for a positive mannitol test, it has not yet been tested in COVID-19 patients and may potentially cause or exacerbate cough or bronchospasm in susceptible individuals in this specific population	(such as in asthma) or presence of signs of significant bronchospasm on examination will be excluded from study participation (Exclusion criterion 5) For the first 10 participants treated before the first IDMC safety review (Section 9.6), RESP301 will be administered by intermittent inhalation until the intended dose is delivered (Section 6.1), so that the treatment can be quickly adjusted and rescue bronchodilator initiated if needed (Section 6.5.1) After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by continuous inhalation.
Study Procedures		
Nebulization	The delivered dose of RESP301, estimated to be ~40% of the masses listed in Table 6-2, can be affected by patient technique and inspiratory capacity leading	Training will be provided to study staff to minimize variation in dose

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	to inter- and intra-patient variation in actual doses of these ingredients, given the acute nature and high disease burden in this proposed clinical setting	
Other		
Risk of spreading SARS-CoV-2	SARS-CoV-2 is known to be transmissable via respiratory droplets and any interventions that may potentially increase cough or aerosolization of respiratory secretions of an infected patient may increase risk of spread of disease	Proper personal protective equipment including appropriate mask, face shield, gown and gloves should be used at all times. When possible, the site staff should not remain in the room during study intervention administration (providing that continuous O_2 monitoring can be conducted remotely and study intervention can be administered correctly)

2.2.2 Benefit Assessment

To date, no treatment of COVID-19 has demonstrated clinical efficacy. Patients and their physicians are critically in need for treatments that decrease the risk of severe levels of disease, particularly the rate of intubation or other ventilatory support as they significantly lead to fatal outcomes. Considering the current pandemic, even a limited improvement in the rate of progression to severe stage of the disease would provide sizable benefits for patients and society.

Nitric Oxide is already marketed (e.g., INOmax®, NOXIVENT™, etc) in the United States (US) and other countries as a gas for continuous use in preterm and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension in conjunction with ventilatory support and other appropriate agents.

It is well tolerated, although under continuous use, a small number of cases of methemoglobinemia have been reported (NOXIVENT™ FDA Prescribing Information).

Nitric oxide has been shown in vitro to inhibit the replication cycle of severe acute respiratory syndrome coronavirus (Akerström S et al, 2005). In addition, NO is a naturally occurring and potent antimicrobial agent in the human body, which is active against viruses, bacteria, fungus, and yeasts (Fang, 1997). In particular, NO inhibits replication in vitro in a number of respiratory viruses including influenza A and B (Rimmelzwaan et al, 1999, Regev-Shoshani et al, 2013), human rhinovirus (Sanders et al, 1998), and respiratory syncytial virus (Ali-Ahmad D et al, 2003).

The ability of RESP301 to have a marked antibacterial action is highly relevant in the context of treating patients with SARS-CoV-2 infection since superimposed bacterial infection is a critical factor and a major cause of morbidity and mortality. RESP301 would restore and replenish the NO deficiency in patients who have succumbed to SARS-CoV-2 infection. RESP301 also has key advantages over inhaled NO gas, as discussed in Section 2.1.

A vibrating mesh nebulizer is advised for administration of RESP301. Amongst the suitable nebulizers, the device chosen for the study is a commercially available, CE-marked vibrating mesh device that delivers a mist of fine droplets in the size range required for pulmonary deposition (Mass Median Aerodynamic Diameter < 5 μm). This has been shown to be ideally suited for penetration deep into the alveolar spaces of the lung. As with all nebulizers in clinical use, there is a residual amount of drug that remains in the nebulizer so that not the full amount of drug is administered. The device operates continuously once initiated and automatically switch off once the medication has been delivered. The device may be used in pediatric and adult populations, as permitted by the prescribed medication, and is suitable for use in home environments or hospital/clinic settings.

As testing inefficient product would distract sites from participating in other research efforts, two interim analysis for futility purpose have been planned.

2.2.3 Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to participants in this first-in-man study, the potential risks identified in association with RESP301 are justified by the anticipated benefits that may be afforded to participants with COVID-19.

3 Objectives and Endpoints

Table 3-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 4 in the modified World Health Organization (WHO) ordinal scale into levels >4	• Proportion of participants who progress to level >4 of modified WHO ordinal scale due to COVID-19 by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO_2	• Change in room air SpO_2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement to a lower level (<4) of modified WHO ordinal scale • Time to progression to a higher level (>4) of modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	

• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements
	• Physical examinations
	• Vital signs
	• Concomitant medications
	• Counts and cumulative incidence of:
	  o Adverse events (AEs)
	  o Serious adverse events (SAEs)
	  o Severe AEs

Objectives	Endpoints

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

4 Study Design

4.1 Overall Design

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study in hospitalized participants with COVID-19 requiring supplemental oxygen (WHO ordinary scale level 4) using a sequential adaptive design to evaluate the efficacy and safety of RESP301 added to standard of care (SOC). Approximately 300 adult (male and female) participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a vibrating mesh nebulizer TID for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

The study will be divided into the following periods:

- Screening period: starts up to 2 days (48 hours) prior to and extends up to the day of treatment initiation (Day 1); Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28 (±2 days)
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±2 days)

After screening, eligible participants will be randomized to either RESP301+SOC or SOC alone on Day 1, and the study treatment will be initiated as applicable (Table 1-1). Screening and randomization may happen on the same day (Day 1).

An individual administration of study intervention will be stopped, and supplemental oxygen resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Section 4.2 and Section 8.1.2). However, the participant may continue with subsequent doses if the Investigator judges that the benefit / risk ratio remains positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored. Inhalation of study intervention should be completed within 20 minutes. A staggered dosing approach will be used for the first 10 participants (up to the first Independent Data Monitoring Committee [IDMC] safety review) to monitor tolerability and response to RESP301 (see Sections 6.1 and 9.6).

The study treatment will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale;
- Experiences an event that requires permanent discontinuation as described in Section 7.1.

In the above cases, participants will be considered as ongoing in the study until the final Safety follow-up phone call (Day 28). Participants will be withdrawn from the study prior to Day 10 (Early withdrawal) only if they withdraw consent.

The total study duration for a participant from screening to last follow up will be up to 30 days (±2 days).

Participants will be stratified by country and presence of risk factor(s) for severe outcomes of COVID-19, based on comorbidities and age, as detailed in Section 6.3.

Two interim analyses are planned (see Section 9.5 for details).

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

4.2 Scientific Rationale for Study Design

NO is a naturally occurring and potent antimicrobial agent in the human body which has been shown in vitro to inhibit replication of severe acute respiratory syndrome coronavirus

(Akerström S et al, 2005), including SARS-CoV2, and other respiratory viruses. NO as an inhaled gas is already marketed in the US and other countries (see Section 2.2.2) but has disadvantages over NO produced locally in the oropharynx or lung airways (lengthy treatment requiring NO canisters, the inhaled NO is expelled in exhalation, the NO gas oxidizes in air to form toxic NO_2 which is a potential lung irritant and a contaminant in the patient's environment, and the half-life of NO in air is short).

RESP301 has potential advantages over inhaled NO gas:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

In this study, the effect of RESP301 as an add on treatment to SOC will be evaluated for its efficacy in reducing rate of progression to a more severe level of COVID-19 and for safety by comparison with SOC alone in hospitalized COVID-19 patients. A sequential adaptive design was chosen in order to assess futility and sample size after interim analyses (see Section 9.5). The study design was developed in accordance with the latest WHO and regional regulatory agencies guidelines.

Several risks factors for severe or fatal outcomes have been reported (Bialek et al, 2020; Huang et al, 2020; Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020; Rong-Hui et al, 2020; Zhou et al, 2020). To minimize bias, the results will be stratified according to the known risk factors as described in Section 6.3.

In this study, measures will be in place to trigger discontinuation of study intervention at a SpO_2 limit pre-agreed at the site (see Section 8.1.2). There is no critical level of oxygen saturation below which tissue hypoxia occurs due to the large number of variables that contribute to hypoxia at the tissue and cellular level (temperature, pH, tissue blood flow). As a result, there is no consensus about what constitutes normal and abnormal oximetry (ATS/ACCP, 2003). To support and guide the team in charge of study intervention administration, a default value of SpO_2=89% and heart rate (120 beats/min) has been proposed as a suitable limit of tolerance. However, for the reason stated above, this limit can be overruled by the Investigator in either direction.

Newly released guidelines for ongoing clinical trials during the COVID-19 pandemic have emphasized the need to reduce the burden on sites and clinical trial personnel/investigators whether they be administrative, site visit, or other burdens (ACRO, 2020; EMA, 2020; HRS, 2020). Thus, without blinding and a placebo, along with minimal procedures, the additional burden on clinical staff has been reduced as much as possible.

4.3 Justification for Dose

Studies with nebulized sodium nitrite (AIR001) in healthy subjects and in patients have shown that inhaled nitrite produces dose proportional plasma pharmacokinetic without accumulation following repeated administration, with low systemic blood levels of nitrite and methemoglobin (<3%). Inhaled nitrite at doses up to 90 mg TID displayed a good safety profile and is well tolerated (Rix et al, 2015; Parsley et al, 2013; Simon et al, 2016).

In this study, 6.0 mL RESP301 (delivered dose 62 mg) will be administered via a vibrating mesh nebulizer, TID with at least 6 hours between each dose. Each 6.0 mL dose for nebulization contains the masses described in Table 6-2.

4.4 End of Study Definition

A participant is considered to have completed the study if he/she has completed the Treatment Period and the Follow-up Period (through the Day 28 EoS Follow-up). End of the study is defined as the last participant's last visit or follow up call.

5 Study Population

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

The study population will consist of male and female COVID-19 patients, including pregnant women and women of child bearing potential. Participants must be able to provide written consent and meet all the inclusion criteria and none of the exclusion criteria.

5.1 Study Rationale

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 plus SOC versus SOC alone in hospitalized patients with COVID-19 requiring supplemental oxygen (WHO level 4).

Each constituent (see Section 6.2) of RESP301 is used as SOC for various conditions. Product application is straightforward, requiring a few minutes of inhalation using a standard nebulizer. RESP301 is likely to be beneficial in treating patients with COVID-19 who are not receiving ventilation but are using supplementary oxygen in order to maintain a safe level of SpO_2.

Considering the well-established and global use of NO and the device, and the current public health emergency resulting from the COVID-19 pandemic, a Phase2/3 study of RESP301 is considered reasonable in order to generate efficacy data in patients infected with COVID-19.

5.2 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant is ≥18 years of age, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participant has laboratory-confirmed SARS-CoV-2 infection as determined by reverse transcriptase polymerase chain reaction (RT-PCR) or other approved clinical testing prior to randomization.
3. Participant is hospitalized in relation to COVID-19, requiring supplemental oxygen to maintain SpO_2 at a safe level (WHO level 4).

Sex

4. Participant is male or female. All females of childbearing potential, including pregnant females, must consent to urine pregnancy testing at screening to be eligible for the study. (Females who are not of childbearing potential do not need to undergo a pregnancy test at screening).

Informed Consent

5. Participant is capable of giving signed informed consent as described in Appendix 1 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.3 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Rapidly deteriorating or likely to require escalation to high flow oxygen, invasive or non-invasive ventilatory support within 24 hours according to Investigator's opinion.
2. Unable to safely receive a nebulized treatment for approximately 4 minutes according to Investigator's opinion.
3. Unable to receive or considered ineligible for invasive or non-invasive ventilatory support.
4. History of methemoglobinemia.
5. Uncontrolled asthma or history of severe bronchospasm.
6. Severe (requiring baseline oxygen therapy > 12 h/day prehospitalization) chronic respiratory disease (e.g., known COPD, pulmonary arterial hypertension, idiopathic pulmonary fibrosis, interstitial lung disease).
7. Suspected or confirmed untreated, active tuberculosis.
8. Severely immune-compromised participants in Investigator's opinion.
9. Recent (within 3 months) active coronary artery disease or decompensated heart failure (New York Heart Association class 3-4).
10. Presence of tracheostomy.

Prior/Concomitant Therapy

11. Chronic (≥4 weeks) use of corticosteroids >10 mg/day of prednisone or equivalent within 4 weeks of randomization.

Prior/Concurrent Clinical Study Experience

12. Participation in other clinical investigations utilizing investigational treatment or within 30 days / 5 half-lives whichever is longer.

Diagnostic Assessments

13. Clinically significant abnormalities in clinical chemistry or hematology at screening, defined as:

- Platelet count <50,000 mm^3
- Alanine aminotransferase or aspartate aminotransferase >5 × upper limit of normal (ULN).
- Estimated glomerular filtration rate <30 mL/min/1.73 m^2 (modification of diet in renal disease formula) or requiring hemofiltration or dialysis.

Other Exclusions

14. Anticipated transfer to another hospital which is not a study site during the treatment period.
15. Allergy to any of the components of the study intervention.

5.4 Lifestyle Considerations

No restrictions are required.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography and reason for screen failure (e.g., eligibility requirements failed).

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

5.5.1 Screening and Enrollment Log and Participant Identification Numbers

The participant's enrollment will be recorded in the Screening and Enrollment Log.

Upon enrollment, each participant will receive a unique participant identification number. Participant numbers must not be re-used for different participants.

6 Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1 Study Intervention Administered

Table 6-1 Study Intervention(s) Administered

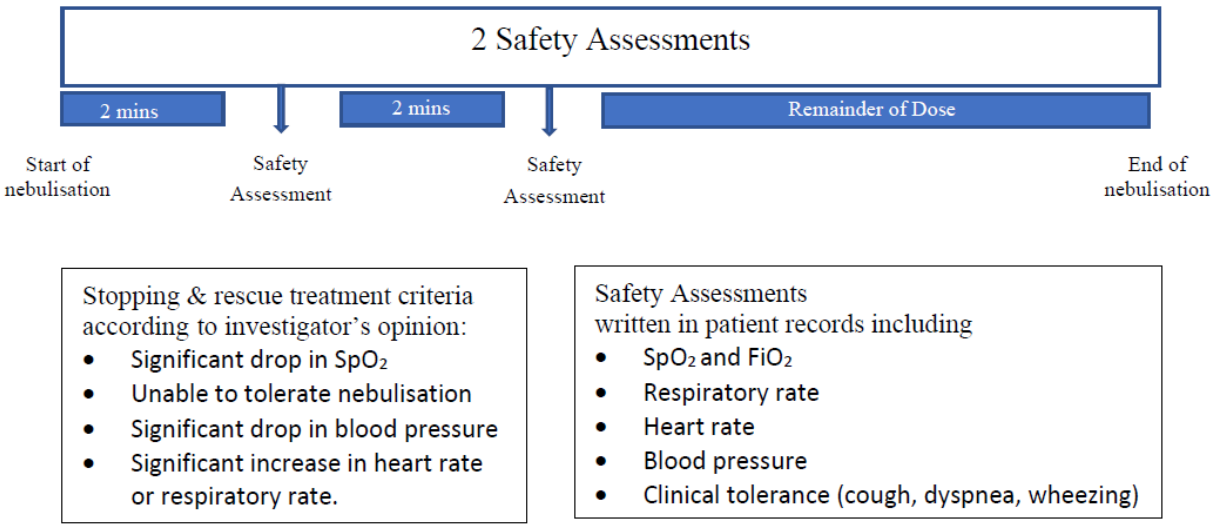
Study Treatment	RESP301 + SOC
Intervention Name:	RESP301
Type:	Drug
Dosage Formulation:	Admixture for inhalation
Unit Dose Strength(s):	Delivered dose (62 mg) sodium nitrite ($NaNO_2$) (6 mL of NO-generating admixture of 150 mM $NaNO_2$, 50 mM mannitol and 100 mM citric acid)
Dosage Level(s):	One 6 mL-inhalation TID (every 8 hours) with at least 6 hours between 2 consecutive doses.
Route of Administration:	Inhalation
IMP and NIMP:	IMP
Sourcing:	RESP301 provided centrally by the Sponsor.
Dosing Instructions:	A vibrating mesh nebulizer will be used to administer the RESP301, under the direction of the study physician. Treatment is to commence within 5 minutes of RESP301 preparation. For the first 10 participants (up to the first IDMC safety review) RESP301 will be administered by intermittent inhalation to monitor tolerability and response to RESP301. After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by a continuous inhalation. Before initiating each RESP301 inhalation, supplemental oxygen will be interrupted for a few minutes until SpO_2 levels are stable. Depending on the extent of SpO_2 decrease, the RESP301 inhalation may be initiated or supplemental oxygen may be resumed at the discretion of the investigator. After treatment initiation, in case of SpO_2 decrease, RESP301 inhalation may be paused and supplemental oxygen resumed. If the participant is unable to tolerate nebulization without supplemental oxygen, nasal oxygen may be administered during nebulization.
Packaging and Labeling:	Study intervention will be provided as two separate vials: $NaNO_2$/mannitol (3 mL) and citric acid buffered to pH 5.4 (3 mL), which will be labeled as required per country requirement.

Staggered dosing approach for the first 10 participants:

For the first 10 participants treated before the first IDMC safety review (Section 9.6), RESP301 dose will be administered in a staggered approach: after 2 minutes of inhalation, treatment is

briefly paused to assess the participant. Provided there is no evidence of bronchospasm, treatment is continued for a further 2 minutes, after which treatment is again briefly paused for participant assessment. Thereafter, provided there is no evidence of bronchospasm and the participant is able to continue, the dose is completed (Figure 6–1), so that treatment can be quickly adjusted and rescue bronchodilator initiated if needed (Section 6.5.1). After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by a continuous inhalation.

Figure 6–1: Staggered Dosing Approach for the First 10 Participants



6.1.1 Medical Devices

1. Medical device (not manufactured by or for Thirty Respiratory Limited) provided for use in this study will be an FDA-approved and CE marked vibrating mesh nebulizer.
2. Instructions for medical device use will be provided in the User Manual.

6.1.2 Device Training

Training will be provided to ensure all study staff are familiar with the device, including mixing the solutions, adding the admixture to the device and cleaning the device.

6.2 Preparation, Handling, Storage, and Accountability

6.2.1 Preparation of Study Intervention Product

RESP301 is prepared at bedside by mixing two sterile 3 mL solutions (one of $NaNO_2$/mannitol and one of citric acid buffered to pH 5.4). The solution is to be nebulized immediately or within

5 minutes post mixing as per the instructions given in the SoA (Table 1-1) and, for the first 10 participants, as in Section 6.1 above (staggered dosing). The first administration of study intervention should be provided under medical supervision.

RESP301 is mixed in the nebulizer chamber before use following the steps below (see Table 6-2 for details of composition):

1. First the vial marked A ($NaNO_2$ and mannitol) is opened and its contents are poured into the nebulizer chamber.
2. Next, the vial marked B (citric acid) is opened and its contents are poured into the nebulizer chamber.
3. The nebulizer lid should be closed, and the nebulizer should be swirled gently for a few seconds to mix the two solutions. The nebulizer should not be inverted.

The inhalation of the medication should start within 5 minutes of mixing the solutions and the inhalation process should be complete within 20 minutes of starting of inhalation.

This combined mildly acidified nitrite solution (6 mL) is inhaled through the mouth using a vibrating mesh nebulizer under the direction of a physician. The purpose of the acidified nitrite aerosol is to deliver NO to the participant. The delivered dose of RESP301, assessed via simulated tidal breathing is estimated to be ~40% of the masses listed below; it should be noted that delivered dose can be further affected by participant's breathing pattern and inspiratory capacity and so inter- and intra-participant variation in actual doses of these ingredients may vary, given the acute nature and high disease burden in this proposed clinical setting.

Table 6-2 Composition of Drug Product, Diluent and Admixture

	Material	Function	Quantity per vial
Drug Product	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Diluent	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Acidified Drug Product Mixture (RESP301)	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**6.0 mL**

6.2.2 Storage and Accountability of Study Intervention

The Investigator or designee must document whether appropriate temperature conditions (<25°C) have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (e.g., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study intervention are provided in the Investigator Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an interactive voice response system (IVRS)/interactive web response system (IWRS). The site will contact the IVRS/IWRS prior to the start of study intervention

administration for each participant. The site will record the intervention assignment on the applicable case report form (CRF), if required.

Potential bias will be reduced using a central stratified randomization to assign participants into one of the study treatment arms, RESP301+SOC or SOC (control), with a randomization ratio of 2:1. Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as follows:

1. Age >65 years
2. Ongoing or currently treated diabetes mellitus
3. Ongoing or currently treated hypertension
4. Ongoing or currently treated cardiovascular disease
5. Ongoing or currently treated chronic lung disease
6. Cancer history of less than 3 years, basal cell skin carcinoma excluded
7. Ongoing or currently treated chronic kidney disease

Participants will be stratified as no risk factor (none of the above criteria), one risk factor (one single of the above) or high-risk factor (2 or more of the above).

6.4 Study Intervention Compliance

Participants will receive study intervention directly from the Investigator or designee, under medical supervision. The date and time of each inhalation will be recorded in the source documents and recorded in the CRF. The study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Concomitant Therapy

During the study, participants will receive institutional SOC for the treatment of COVID-19.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency and route

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1 Rescue Medication

For participants treated before the first safety review by the IDMC (Section 9.6), at the time of RESP301 treatment, a ready-to-use nebulization of a bronchodilator (short acting beta agonist such as salbutamol/albuterol according to investigational site standard practice for acute bronchospasm) may be administered to participants in case of bronchospasm.

After reviewing safety data, the IDMC will decide whether the availability of rescue bronchodilator at the time of RESP301 treatment is still required.

Although the use of rescue medications is allowable for the first 10 participants, the use of rescue medication should be delayed, if possible, for at least 2 minutes following each administration of study intervention. The date and time of rescue medication administration as well as the name of the rescue medication must be recorded.

6.6 Intervention After the End of the Study

After the end of the study, participants may continue their SOC (if any). Study intervention for COVID-19 will not continue beyond this study.

7 Discontinuation of Study Intervention and Participant Discontinuation

7.1 Permanent Discontinuation of Study Intervention

It may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the study to be evaluated for the Day 14 and Day 28 safety follow-ups. See the SoA (Table 1-1) for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

Study intervention will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale.

Additionally, study intervention should be permanently discontinued in the following circumstances:

1. Discontinuation of study intervention for abnormal liver tests should be considered if the Investigator believes that it is in best interest of the participant.

2. If, as part of normal clinical follow-up, a clinically significant change in ECG is identified, the Investigators should exercise their clinical judgement to decide whether continuing study intervention administration is in the best interest of safety of the participant.

3. AE/SAE if the Investigator believes that it is in best interest of the participant, including the following:

- Development of significant bronchospasm * or worsening of cough that is not tolerated by the participant and leads to immediate discontinuation of the nebulization.

- Progressive COVID-19 requiring initiation of non-invasive or invasive ventilation or high flow oxygen.

- Development of mHb level above 3%.

- Inability to safely continue receiving study intervention due to compromised respiratory status while off supplemental oxygen and receiving nebulized therapy (manifest by drop in SpO_2, increased respiratory rate or clinical other findings).

Refer to the SoA (Table 1-1) for data to be collected at the time of study intervention discontinuation and follow-up and for any further evaluations that need to be completed.

** Inhaled citric acid and inhaled mannitol provoke bronchospasm in patients with bronchial hyperresponsiveness in a dose dependent pattern. Although the concentrations of mannitol and citric acid are below the provocative dose and concentration inducing bronchospasm, there is a theoretical risk that a participant may develop acute bronchospasm during the use of RESP301. Participants should therefore be monitored during RESP301 administration. In case of clinical intolerance or saturation decrease, nebulization should be immediately discontinued (see Section 7.2). Further clarification of the differences in signs and symptoms for acute bronchospasm and COVID-19 progression are provided in* Table 7-1.

Table 7-1 Differences in Signs and Symptoms for Acute Bronchospasm and COVID-19 Progression

	Acute bronchospasm related to inhaled bronchial irritant	Progression of COVID-19 lung disease to acute respiratory failure
Mechanism	Acute contraction of bronchial muscles resulting in bronchial obstruction resulting in increased ventilatory load	Cytokine storm resulting in alveolar damage resulting in gas transfer impairment
Onset	Within minutes (<15 minutes) following inhalation (no late phase since it is not an allergic IgE mediated reaction)	Within hours or days and not related to nebulization
Clinical auscultatory signs	Wheezing (almost all cases)	Bilateral rales (inconstant)
Patient history	Asthma or respiratory allergy	Age > 65 years, increased BMI, high blood pressure, cardiac diseases, etc.
Reversibility with inhaled beta mimetics	Within minutes	No

Abbreviations: BMI=body mass index; IgE=immunoglobulin E.

7.2 Temporary Discontinuation of Study Intervention

Brief temporary discontinuation of study intervention is permitted during the study, providing inhalation of study intervention is completed within 20 minutes.

Study intervention should be temporarily discontinued in the following circumstance:

1. Clinical intolerance to nebulization.

   An individual administration of study treatment will be stopped, and supplemental oxygen resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Sections 4.2 and 8.1.2).

The participant may continue with subsequent doses if the Investigator judges that the benefit / risk ratio remains positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored.

Refer to Section 6.1 for information on staggered dosing in the first 10 participants.

7.3 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or

administrative reasons. The participant will be definitively discontinued from both the study intervention and from the study at that time.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

At the time of discontinuing from the study, if possible, an early discontinuation assessment should be conducted, as shown in the SoA (Table 1-1).

7.4 Loss of Participants to Follow-Up

A participant will be considered lost to follow-up if he or she is unable to be contacted by the study site. The following actions must be taken if a participant cannot be contacted at Day 14 or Day 28:

- The site must attempt to contact the participant and counsel the participant on the importance of maintaining the assigned schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- In cases in which the participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record/CRF.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 Study Assessments and Procedures

Study procedures and their timing are summarized in the SoA (Table 1-1). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Table 1-1).

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 20 mL.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Informed Consent

Informed consent must be documented according to Appendix 1, Section 10.1.3.

Eligibility Criteria

All inclusion and exclusion criteria will be reviewed by the Investigator or designee to ensure that the participant qualifies for the study.

Participant Identification Card

All participants will be given a participant identification card identifying them as participants in a research study. The card will contain study site contact information (including direct telephone numbers) to be used in the event of an emergency. The Investigator or qualified designee will provide the participant with a participant identification card immediately after the participant provides written informed consent. At the time of intervention allocation/randomization, site personnel will add the intervention/randomization number to the participant identification card.

Medical History

A medical history will be obtained by the Investigator or qualified designee. The medical history will collect all active conditions and any condition diagnosed that the Investigator considers to be clinically relevant. Details regarding the disease for which the participant has enrolled in this study will be recorded separately and not listed as medical history.

Prior and Concomitant Medications Review

The Investigator or qualified designee will review prior medication use and record prior medications taken by the participant within 3 months prior to screening. This should include a history of hypertension medication, in particular angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

The Investigator or qualified designee will record medication, if any, taken by the participant during the study through the last assessment. Concomitant medications will be recorded on an ongoing basis during the study (or longer if related to an SAE).

8.1 Efficacy Assessments

8.1.1 Modified WHO Ordinal Scale

A modified WHO ordinal scale will be used for consistency with the recent study of lopinavir-ritonavir in adults hospitalized with severe COVID-19 (Cao et al, 2020), to record the participant's status at the time of assessment. The modified WHO ordinal scale includes the following levels:

1. Not hospitalized, no limitations on activities;
2. Not hospitalized, limitation on activities;
3. Hospitalized, not requiring supplemental oxygen;
4. Hospitalized, requiring supplemental oxygen;
5. Hospitalized, on non-invasive ventilation or high-flow oxygen devices;
6. Hospitalized, on invasive mechanical ventilation or extra corporeal membrane oxygenation (ECMO);
7. Death.

8.1.2 Pulse Oximetry

Pulse oximetry measurements will be performed to evaluate SpO_2 as outlined in the SoA (Table 1-1) and in accordance with the site standard operating procedures, on a medical-grade medical device. Measurements will be taken with a probe on fingertip or earlobe and recorded as percent oxygenated hemoglobin. Supplemental oxygen used at the time of assessment and method of oxygen delivery will be collected and documented along with the SpO_2.

Prior to administration of study intervention, supplemental oxygen will be held for at least a minute and SpO_2 checked in order to ensure participant may safely receive the study intervention. For safety reasons, since due to the current pandemic it cannot be guaranteed that study intervention will be consistently administered under direct medical supervision, the study Investigators should provide study personnel who are able to monitor the participant and their SpO_2 and ensure that should it decrease to a threshold, this would trigger immediate discontinuation. By default, unless overruled by the study Investigator, this SpO_2 threshold is set to 89%. This threshold should be entered into the saturation monitoring device to trigger an alarm during nebulization.

FiO_2 will be measured per site standard practice and recorded in the eCRF. The device used to administer oxygen should also be recorded.

8.1.3 National Early Warning Score (NEWS) 2

The NEWS 2 will be measured prior to study intervention in the morning according to the SoA in Table 1-1.

The NEWS is based on a simple aggregate scoring system in which a score is allocated to physiological measurements, already recorded in routine practice, when patients present to, or are being monitored in hospital (RCP, 2012). Six simple physiological parameters form the basis of the scoring system:

1. Respiration rate
2. Oxygen saturation
3. Systolic blood pressure
4. Pulse rate
5. Level of consciousness or new confusion*
6. Temperature

**The patient has new-onset confusion, disorientation and/or agitation, where previously their mental state was normal – this may be subtle. The patient may respond to questions coherently, but there is some confusion, disorientation and/or agitation. This would score 3 or 4 on the Glasgow Coma Scale (rather than the normal 5 for verbal response), and scores 3 on the NEWS system.*

A score is allocated to each parameter as they are measured, with the magnitude of the score reflecting how extremely the parameter varies from the norm (zero for ‘normal’; maximum

score 3). The score is then aggregated. The score is increased by 2 points for people requiring supplemental oxygen to maintain their recommended oxygen saturation. This is a pragmatic approach, with a key emphasis on system-wide standardization and the use of physiological parameters that are already routinely measured in National Health Service (NHS) hospitals and in prehospital care, recorded on a standardized clinical chart – the NEWS 2 chart (Refer to Appendix 2: The NEWS 2 Scoring System).

Reproduced from: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017 (RCP, 2017).

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1-1).

8.2.1 Physical Examinations

A complete physical examination will include, at a minimum, assessments of the head/ear/eyes/nose/throat, cardiovascular, respiratory, gastrointestinal, lymphatic, skin and neurological systems. Height (screening only) and weight will also be measured and recorded.

A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

8.2.2 Vital Signs

Vital signs to be collected as outlined in the SoA (Table 1-1) and include body temperature, heart rate, blood pressure, and respiratory rate.

Body temperature will be assessed per the local practice (temporal or otic are preferred sites), and site will be recorded. Pulse rate, respiratory rate, and blood pressure will also be assessed per site SOC. Where possible the same methods should be used throughout the study for an individual participant.

Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones). During study intervention administration, an alarm threshold should be set for heart rate at 120 beats/min (or as indicated by the Investigator), as outlined in the SoA (Table 1-1).

Vital signs (to be taken before blood collection for laboratory tests) will be measured after the participant has been sitting for 5 minutes.

8.2.3 Electrocardiograms

Single 12-lead ECG will be obtained as outlined in the SoA (Table 1-1) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals for local reading.

8.2.4 Clinical Safety Laboratory Assessments

Refer to Appendix 3 for the list of clinical laboratory tests to be performed and to the SoA (Table 1-1) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically relevant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically abnormal during participation in the study or within 4 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, where possible, and the Sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the Laboratory Manual and the SoA (Table 1-1).
- If laboratory values from laboratory assessments not specified in the protocol and performed at the institution’s local laboratory result in the need for a change in participant management or are considered clinically relevant by the Investigator (e.g., are considered to be an SAE or an AE), then the results must be recorded in the CRF.

8.3 Adverse Events and Serious Adverse Events

The definitions of AEs and SAEs can be found in Appendix 4.

Adverse events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following

up AEs that are serious, considered related to the study intervention or the study, or that caused the participant to discontinue the study intervention (see Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from signing of informed consent until the last follow-up visit at the time points specified in the SoA (Table 1-1).

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours of the Investigator becoming aware of the SAE, as indicated in Appendix 4. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obliged to actively seek AEs or SAEs after the conclusion of study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.3.2 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is given in Appendix 4.

8.3.4 Regulatory Reporting Requirements for SAE

Prompt notification (within 24 hours, see Appendix 4) by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and Investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAE) from the Sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

All women, including women of childbearing potential are allowed in the study. Urine pregnancy test will be performed on female participants of childbearing potential and pregnant females at screening. Females who are not of child bearing potential do not need to undergo a screening pregnancy test.

Pregnant females will be followed to determine the outcome of the pregnancy:

- The Investigator will collect any follow-up information on the participant and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of the pregnancy will be reported, regardless of fetal state (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered to be SAEs. Any post-study pregnancy-related SAE considered reasonably related to study intervention by the Investigator will be reported to the Sponsor as described in Section 8.3.4. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related events (DREs) are common in participants with COVID-19 and can be serious/life-threatening:

- Fever
- Cough*
- Dyspnea*
- Asthenia
- Loss of sense of taste and smell

** A cough or dyspnea episode related to study intervention administration does not meet the definition of a DRE and should be reported as an AE.*

Because these events are typically associated with the disease under study, they will not be reported as AEs.

NOTE: However, if either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the Investigator's opinion, of greater intensity, frequency, or duration than expected for the individual participant.

  OR

- The Investigator considers that there is a reasonable possibility that the event was related to study intervention.

8.3.7 Adverse Events of Special Interest (Not Applicable)

No AEs of special interest are defined for this study.

8.3.8 Medical Device Deficiencies (Not Applicable)

The medical device to be used in this study is a vibrating mesh nebulizer that is FDA-approved and CE marked and is to be used per the manufacturer's recommendations. Therefore, data on device deficiencies will not be collected in this study.

8.4 Tokenization (Optional)

Tokenization is the process of converting a piece of data into a random string of characters known as a token. Tokenization protects sensitive data by substituting non-sensitive data. The

token serves merely as a reference to the original data, but cannot be utilized to determine those values. The advantage of tokens is that there is no mathematical relationship to the real data that they represent. The real data values cannot be obtained through reversal, and hence, a breach renders the information invaluable. Tokens are being increasingly used to secure varying types of sensitive information. In particular, personal identifiable information such as healthcare information, email addresses and account numbers are such examples. From a security perspective, tokenization significantly reduces risk based on the fact that sensitive data cannot be breached if it is not there in the first place.

Tokenization applies only to US participants who agree and sign the optional ICF. The piece of personal data needed to generate the token will be collected to create a unique, de-identified token. This token would be instrumental to enrich and aggregate other study participants' data coming from different sources for the purpose of future research. The benefit of linking data in general, is that the data set that is created can be used to answer a variety of healthcare- and therapy-related questions that could not otherwise be answered through conventional means.

8.5 Treatment of Overdose (Not Applicable)

There is no risk of overdose.

8.6 Pharmacokinetics (Not Applicable)

Pharmacokinetic parameters are not evaluated in this study.

8.7 Pharmacodynamics (Not Applicable)

Pharmacodynamic parameters are not evaluated in this study.

8.8 Genetics (Not Applicable)

Pharmacogenomics are not evaluated in this study.

8.9 Biomarkers (Not Applicable)

Biomarkers are not evaluated in this study.

8.10 Medical Resource Utilization and Health Economics (Not Applicable)

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 Statistical Considerations

9.1 Statistical Hypothesis

RESP301 reduces the rate of progression to level 5 and above in the modified WHO ordinal scale in COVID-19 (see Section 8.1.1).

This represents a composite endpoint of (i) death (level 7), (ii) hospitalized, on invasive mechanical ventilation or ECMO (level 6), or (iii) hospitalized, on non-invasive ventilation or high-flow oxygen devices (level 5).

9.2 Sample Size Determination

A total of 300 hospitalized participants with confirmed COVID-19 will be randomized 2:1 to receive RESP301+SOC (200 participants) or SOC alone (100 participants).

The comparison between the treatment arms for the primary endpoint will have approximately 80% power and alpha level 0.025 (one-sided) to demonstrate significant reductions (15% versus 30%) of the primary endpoint between RESP001+SOC and the Control arm (SOC), a 50% relative reduction for the proportion of participants who progress to level >4 (see definition of the primary endpoint).

Two interim analyses are planned (see Section 9.5 for details):

1. The first IDMC interim analysis will take place after the first 60 participants have completed Day 10 of the study to evaluate whether the study can be stopped for futility based on change from baseline in room air SpO_2. For a final decision to stop the study for futility the results on other endpoints will be considered as well.
2. The second interim analysis will take place after 150 participants have completed Day 14 post-randomization based on event rate for the primary endpoint. The purpose of the second interim analysis will be futility as well as a potential sample size re-estimation in case the actual results differ from the original assumptions.

In addition to the review of efficacy data, safety will be assessed at each of the interim analysis by the IDMC. Further details are provided in Appendix 5.

9.3 Populations for Analyses

For purposes of analysis, the following analysis sets are defined:

Table 9-1 Populations for Analysis

Population (Analysis Set)	Description
Intent-To-Treat (ITT) Population	The ITT Population will include all randomized participants. The ITT participants will be analyzed according to randomized treatment, irrespective of the actual treatment received. Participants who withdraw from treatment early will be followed for the assessment of the Day 14 primary endpoint. All efficacy analyses will be performed using the ITT Population.
Per Protocol (PP) Population	The PP Population will include all participants in the ITT Population with no major protocol deviations that may significantly impact data integrity or patient safety. The PP Population will be used for supportive analyses of the efficacy measurements.
Safety Population (SP)	The SP will include all randomized participants who inhale any amount of study intervention or are randomized to the control arm. The SP will be analyzed according to the actual treatment received. This set will be used for the safety analyses.

The ITT Population will be the primary analysis set for all efficacy analyses and the PP Population will be used to demonstrate robustness of results for the primary efficacy endpoint.

9.4 Statistical Analyses

Below is a description of planned statistical analyses. Further details are presented in the Statistical Analysis Plan (SAP).

9.4.1 General Considerations

All statistical analyses will be conducted using SAS, Version 9.4 or later. Baseline characteristics will be summarized by treatment arm. For continuous measures the mean and standard deviation (SD) will be summarized. Categorical variables will be described by the proportion in each category. In addition, 95% confidence intervals (CIs) will be computed as indicated.

All the categorical variables including the primary endpoint will be summarized by treatment with the numbers and percentages of the participants. Treatment difference will be tested using a Cochran–Mantel–Haenszel test stratified by 1) country and 2) participant's clinical status at Baseline. For the categorical endpoints, relative risk and its 95% CI will be presented.

All of the continuous variables, including the changes from Baseline, will be summarized by treatment with the means, SD, medians and the ranges. The mixed model with repeated

measurements /analysis of covariance model with treatment, country, participant's clinical status at Baseline and visit as the model term, and Baseline value as the covariate will be used to test for the significance of the treatment difference. Least square means, standard errors, 95% CIs and p-values will be presented.

Time to event endpoints will be analyzed using the Cox Proportional Hazard model with treatment, participant's clinical status at Baseline and country as the model term. The hazard ratio of RESP301+SOC versus SOC will be presented along with 95% CI and p-value from the model. The Kaplan-Meier curves of the time to events will be presented by treatment for each applicable endpoint.

Handling of missing data

If participants are discharged from the hospital prior to Day 14 due to improvement of the clinical status and their status on Day 14 cannot be obtained, their status on Day 14 for the primary endpoint will be imputed with the status on the day of discharge. Depending on the reasons for missing data on the primary endpoint up to Day 14, additional sensitivity analyses will be performed. Further details on handling on missing data will be provided in the SAP.

9.4.2 Primary Endpoint

The primary endpoint is the proportion of participants who progress to level >4 of the modified WHO ordinal scale due to COVID-19 by Day 14.

9.4.3 Secondary Endpoint(s)

The key secondary endpoints are:

1. Change from baseline on the modified WHO ordinal scale at each visit up to Day 28
2. Change in room air SpO_2 from baseline over time
3. Change in NEWS 2 symptom score from baseline over time
4. Time to improvement to a lower level (<4) of the modified WHO ordinal scale
5. Time to progression to a higher level (>4) of the modified WHO ordinal scale

Additional secondary endpoints are:

1. Time to hospital discharge
2. Incidence of mortality by Day 28

9.4.4 Tertiary/Exploratory Endpoint(s)

CCI

- CCI

9.4.5 Other Safety Analyses

All safety analyses will be performed on the Safety Population.

1. Safety and tolerability assessed by clinical safety laboratory measurements, physical examinations, vital signs, concomitant medications; cumulative incidence of AEs, SAEs and severe AEs
2. Incidence of participants unable to tolerate nebulization due to:
    - Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
    - Other clinical signs of intolerance according to Investigator's opinion
3. Incidence of clinical bronchial hyper-responsiveness related to nebulization

9.4.5.1 Adverse Events

Adverse Events will be coded using the MedDRA coding dictionary.

The number and percentage of participants with any AE, any related AE, any SAE, any related SAE, any severe AE, and related severe AE as well as the total number of events for each category will be summarized. The number of deaths due to an AE, hospitalization due to an AE, and study discontinuation due to an AE will be summarized.

The number and percentage of participants with an AE, as well as the total number of AEs, will be summarized by SOC and preferred term. This tabulation will be repeated for related AEs, SAEs, related SAEs, severe AEs, and related severe AEs.

All AEs will be provided in patient listings. Patient listings of AEs causing discontinuation of study medication, AEs leading to death, SAEs, related AEs, and severe AEs will be produced.

9.4.5.2 Clinical Laboratory Evaluation

Baseline is defined as the last non-missing value obtained at the screening visit and prior to the first exposure to study drug. Actual values and changes from Baseline clinical laboratory tests

will be summarized by study day. If more than one laboratory result is reported per study visit per parameter, the last non-missing result will be selected for change from Baseline analysis.

Laboratory test results will be classified according to the reference ranges and clinical significance as determined by the Investigator. The number of participants with a non-missing result, the number and percentage of participants with a clinically significant result less than the lower limit of normal, non-clinically significant result more than the ULN, and clinically significant result more than the ULN will be summarized by study visit. If more than one laboratory result is reported per study day per parameter, the result yielding the most severe classification will be selected for analysis.

Categorical laboratory test results (urinalysis excluding pH) will be summarized by study visit. If more than one laboratory result is reported per study day per parameters, the result yielding the most severe classification will be selected for analysis.

Participants who had urine pregnancy test at screening and the results will be listed.

Participants with clinically significant abnormal laboratory test results will be listed. This listing will include all results of the laboratory parameter that was abnormal and determined to be clinically significant by the Investigator for a participant across study visit.

9.4.5.3 Vital Signs

Baseline is defined as the last non-missing value obtained in screening and prior to the first exposure to study drug. Actual values and changes from Baseline in vital signs will be summarized by study day and study time point. All vital sign data will be presented in patient listings.

Vital sign values will be classified according to the clinical significance as determined by the Investigator. The number of participants with a non-missing result, the number and percentage of participants with a non-clinically significant result, and clinically significant result will be summarized by study visit and study time point. If more than one vital sign result is reported per study day and study time point per parameter, the result yielding the most severe classification will be selected for analysis.

Participants with clinically significant vital sign values will be listed. This listing will include all results of the vital sign parameter that was determined by the Investigator to be clinically significant for a participant across study time points.

9.4.5.4 Physical Examination

Abnormal physical examination findings will be listed.

9.4.6 Other Analyses

Other analyses may be added to the SAP as applicable.

9.5 Interim Analyses

Two interim analyses will be performed.

1. The first analysis will be conducted when about 60 participants (40 participants in the RESP301+SOC arm and 20 participants in the SOC arm) have completed the Day 10 assessment. The purpose of this analysis is to evaluate efficacy of RESP301 with the application of a futility criterion based on the results on SpO_2 change from baseline on Day 10. The following futility criterion will be used for this first interim analysis:

   If the difference in the percentage of participants with at least a 2% improvement in SpO_2 between both treatment arms is less than 5%, benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

   As no other modification of the study at the time of the first interim analysis is considered, no adjustment of the alpha level is required.

2. The second interim analysis will be conducted after 150 participants (100 participants in the RESP301+SOC arm and 50 participants in the SOC arm) have completed the Day 14 assessment. The purpose of this analysis is the assessment of futility or a sample size re-estimation in case the actual results differ from the original assumptions for the power calculations of the study, related to the percentage of participants meeting the primary endpoint in the control arm and/or the relative treatment benefit achieved in the RESP301+SOC arm compared to the SOC arm.

   To account for the multiple testing due to the second interim analysis an adjustment for the type I error alpha will be applied using the Haybittle-Peto approach which would spend one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis. The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration

will be given to stop the study for futility. At the same time sample size re-estimation will be performed to achieve 80% power at the end of the study. The methodology for the adjustment and the procedure to maintain the type I error level will be described in greater detail in the SAP.

Detailed information, including the boundaries futility and characteristics for the sample size re-estimation at the time of the interim analyses will be provided in the SAP and Data Monitoring Committee (DMC) Charter. Further details are also provided in Appendix 5.

9.6 Data Monitoring Committee (DMC)

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet to review after 10 and 20 participants have completed as well as at the time of the interim analyses (ie, after 60 and 150 participants have completed; see Section 9.5).

The detailed procedures and criteria of the interim analyses will be described in the DMC Charter.

10 Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
    - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
    - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The Investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC.
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures.
    - Overall conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH GCP guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Financial Disclosure

Information on financial disclosure can be found in the Investigator Site File.

10.1.3 Informed Consent Process

The Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IEC/IRB and signed by all participants subsequently enrolled in the study as well as those currently enrolled in the study.

For US participants who agree to tokenization, a separate optional ICF will be provided.

10.1.4 Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Data Protection for Tokenization information is addressed in the separate optional ICF (for US participants only).

10.1.5 Committees Structure

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study (see Sections 9.5 and 9.6).

10.1.6 Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategies (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for at least 2 years after the last approval of a marketing application or 15 years from completion of the study, whichever is longer according to the relevant local laws and/or regulations. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

- All data generated by the site personnel will be captured electronically at each study center using eCRFs. Data from external sources (such as laboratory data) will be imported into the database. Once the eCRF clinical data have been submitted to the central server at the independent data center, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.
- If additional corrections are needed, the responsible monitor or data manager will raise a query in the electronic data capture (EDC) application. The appropriate staff at the study site will answer queries sent to the Investigator. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will freeze the eCRF page.
- The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the EDC system/eCRF.

10.1.7 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

10.1.8 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the first participant signing the informed consent form and will be the study start date.

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the Investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up

10.1.9 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor at least one month before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.10 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first participant is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/Competent Authority approval prior to implementation (if appropriate). In the US: Following approval, the protocol

amendment(s) will be submitted to the Investigational New Drug application under which the study is being conducted.

Administrative changes (not affecting the participant benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.1.11 Liability and Insurance

10.1.11.1 Access to Source Data

Access to source data is described in the Clinical Site contract.

10.2 Appendix 2: The NEWS 2 Scoring System

Chart 1: The NEWS 2 scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

Abbreviations: CVPU: C=new onset confusion, disorientation or agitation, V=responds to voice, P=responds to pain, U=unresponsive; SpO_2=oxygen saturation.

Source: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2

10.3 Appendix 3: Clinical Laboratory Tests

The tests detailed in Table 1-1 will be performed by the local laboratory.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol. Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 10-1 Protocol-Required Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	CBC without differential: White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet, mean platelet volume Methemoglobin, nitrite			
Clinical Chemistry[1]	Blood urea nitrogen Creatinine Glucose non-fasting	Potassium Sodium Chloride Bicarbonate/CO_2	Aspartate Aminotransferase / Serum Glutamic-Oxaloacetic Transaminase Alanine Aminotransferase/ Serum Glutamic-Pyruvic Transaminase Alkaline phosphatase / Lactate dehydrogenase	Total and direct bilirubin Coagulation (PT/INR, aPTT)
Other Screening Tests	Highly sensitive urine human chorionic gonadotropin pregnancy test (for women of childbearing potential and pregnant women) [2]			

Laboratory Assessments	Parameters
	The results of each test must be entered into the eCRF.

NOTES:

1 Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1. All events of ALT ≥3 × ULN and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

2 Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

Investigators must document their review of each laboratory safety report.

10.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1 Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (e.g., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.4.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization (Applies only during the safety follow up period for participants who may have been discharged during the treatment period, ie, between Day 1 and Day 10)

- In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or intervention that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are considered AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective intervention of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive intervention in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.4.3 Recording and Follow-up of AE and SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event. • The Investigator will then record all relevant AE/SAE information in the CRF. • It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to the clinical research organization (CRO) in lieu of completion of the AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by the CRO. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to Parexel Safety Services for review. • The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories: • Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities. • Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe. An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality
• The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. • A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The Investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated. • The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment. • For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report in the electronic CRF/EDC. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to electronic CRF/EDC.** • The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. • The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE
• The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the DMC to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • New or updated information will be recorded in the originally completed CRF.

- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.4.4 Reporting of SAE

SAE Reporting to Parexel Safety Services via Electronic Data Collection Tool

The Investigator must report any SAEs to the Parexel Safety Services within 24 hours of becoming aware of the event.

All SAEs will be recorded from signing of informed consent until the end of the study. Serious adverse events occurring after the end of the study and coming to the attention of the Investigator must be reported only if they are considered (in the opinion of the Investigator) causally related to study intervention.

- The primary mechanism for reporting an SAE to Parexel Safety Services will be the electronic data collection tool.
- The site will additionally use the paper SAE data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- The Investigator and the Sponsor (or Sponsor's designated agent) will review each SAE report and the Sponsor/Parexel will evaluate the seriousness and the causal relationship of the event to study intervention. In addition, the Sponsor (or Sponsor's designated agent) will evaluate the expectedness according to the reference document (Investigator Brochure or Summary of Product Characteristics). Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by email.
- Contacts for SAE reporting can be found in the Investigator Manual.

Suspected Unexpected Serious Adverse Reactions (SUSARs)
Any AE that is a SUSAR has additional reporting requirements, as described below. • If the SUSAR is fatal or life-threatening, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within seven calendar days after the Sponsor learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information should be reported within an additional 8 days (15 days total). • If the SUSAR is not fatal or life-threatening but is otherwise serious, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within 15 calendar days after the Sponsor learns of the event. The Sponsor will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of participants. Follow-up information may be submitted if necessary. The Sponsor will also provide annual safety updates to the regulatory authorities and IECs responsible for the study. These updates will include information on SUSARs and other relevant safety findings.

All SAEs that occur during the study, and all SAEs occurring up to 18 days after receiving the last dose of study intervention, whether considered to be associated with the study intervention or not, must be reported within 24 hours via electronic data collection tool and paper data collection tool to Parexel Safety Services.

The minimum information required for an initial report is:

Name of person sending the report (e.g., name, address of Investigator);

- Participant identification (screening/randomization number, initials, NOT participant name);
- Protocol number;
- Description of SAE;
- Causality assessment, if possible.

However, as far as possible all points on the SAE form should be covered in the initial report, or the completed SAE form itself must be emailed or faxed to the Parexel Safety Services. In addition, the event must be documented in the electronic CRF/EDC system.

After receipt of the initial report, Parexel Safety Services will review the information and, if necessary, contact the Investigator, to obtain further information for assessment of the event. Parexel will be responsible for all information processing and reporting according to local legal requirements. Where necessary, Investigators will inform regulatory authorities in their own countries.

10.5 Appendix 5: Statistical Design Considerations

10.5.1 Some General Design Considerations

1. Binary primary endpoint: progression to critical stage/death up to Day 14
    - to be analyzed by a Cochran-Mantel-Haenszel (CMH) stratified for the factors used in the stratified randomization
    - subjects who withdraw from study treatment will be followed up until their primary endpoint outcome is known (i.e., up to Day 14 or progression to critical stage/death, whatever is first)
    - it is, therefore, not clear what current protocol synopsis text could mean: *If subjects are discharged from the hospital prior to day 14 due to improvement of the clinical status and their status on day 14 cannot be obtained, their status on day 14 for the primary endpoint will be imputed with the status on the day of discharge. and what the implications on statistical study characteristics could be. Further discussion is needed.*
2. Timing of the two planned interim analyses with stopping options is stated as

There are 2 interim analyses planned, the first interim analysis after the first 60 participants have completed day 10 of the observation period … and a second interim analysis after 150 participants have completed day 14 of the observation period.

With the current text for the timing of the first interim analysis, it is not clear how many subjects will have been randomized at least 14 days prior to the interim data cut-off date and can therefore provide data for the binary primary endpoint ("progression to critical stage/death up to Day 14").

For such a binary endpoint, Parexel does not recommend including any participant randomized less than 14 days prior to the interim data cut-off date (note this would be different to a study with a time-to-event endpoint) as

- for such participants not yet progressed, the outcome up to Day 14 is unknown and should not be imputed as "not progressed up to Day 14"
- for such participants progressed prior to Day 14, the outcome is known but their inclusion would bias the estimation of progression probability up to Day 14 in a upwards direction.

In order to set up the adaptive group-sequential study design, the following assumptions have been used:

- first interim analysis: a total of ≈ 39 participants randomized at least 14 days prior to data cut-off date
- second interim analysis: a total of ≈ 50% of the initially planned number of participants randomized at least 14 days prior to data cut-off date.

3. Non-binding DMC guidance for stopping the study for futility at the first interim analysis is stated as:

If the difference in the percentage of participants with at least a 2% improvement in SpO2 between both treatment arms is less than 5%, a benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

As the main futility stopping criterion is based on improvement in SpO_2 (and not on the primary efficacy endpoint), this first interim analysis will be "ignored" in these statistical considerations for an adaptive group-sequential design. We may revisit the first interim analysis at a later point in time when more information is available.

4. Non-binding DMC guidance for stopping the study for futility at the second interim analysis stated as:

The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration will be given to stop the study for futility.

5. Current DMC guidance for stopping the study for superior efficacy at the second interim analysis is stated as:

To account for the multiple testing due to the second interim analysis an adjustment for the type-I-error probability will be applied using the Haybittle-Peto approach with one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis.

6. For initial sample size calculation, the following additional assumptions / features from the protocol have been used

- progression probability up to Day 14 for Standard of Care (SOC): 30%

- progression probability up to Day 14 for RESP301+SOC: 15%
- desired power assuming the alternative hypothesis stated above: 80%
- 2:1 randomization

7. Method for adaptation of the sample size based on the second interim analysis data is stated as

A sample size re-estimation will be performed to achieve 80% conditional power at the end of the study. The methodology for the adjustment and the procedure to maintain the type-I-error level will be described in greater detail in an appendix to the protocol. The maximum increase of the sample size will be limited to a total number of 600 randomized subjects.

Parexel has put a suggestions with some fictitious interim data for illustration in Section 10.5.3; that section also illustrates by simulations the Cui/Hung/Wang (CWH) approach using a weighted (weights fixed at the design-stage) test for the final analysis versus the Chen/DeMets/Lan (CDL) approach without such weights at the final analysis.

10.5.2 General Design Characteristics

Parexel proposes to base non-binding futility stopping criteria on conditional power criteria. Conditional power (CP) is the conditional probability for achieving statistically significant superiority of RESP301+SOC over SOC at the final analysis, given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study (an alternative would be predictive power (PP), a Bayesian version of CP where prior distributions and observed interim results are combined to obtain the probability for achieving statistically significant superiority of gimsilumab over placebo at the final analysis).

Randomization 2:1	**Group-Sequential Design**
1st Interim Analysis (IA) 2nd IA	N ≈ 39 for D14 ≈ 50% for D14
Futility criterion 1st IA Futility criterion 2nd IA Superior efficacy criterion 2nd IA	Based on SpO_2 CP < 4% p < 0.0005
Total sample size required for 80% power	**300**
Futility stopping probability at 2nd IA under "No effect" Futility stopping probability at 2nd IA under "Planned effect 30% vs 15%"	1st IA: **NA** 2nd IA: **70%** 1st IA: **NA** 2nd IA: **6%**
Superiority stopping probability at 2nd IA under "Planned effect 30% vs 15%" Superiority stopping probability at 2nd IA under "30% vs 10%"	**10%** **28%**

10.5.3 Adaptive Sample Size Re-Estimation

Another objective of the 2nd interim analysis is to re-estimate the sample size based on the unblinded interim results.

Proposed procedure at 2nd interim analysis (planned to include approximately 150 subjects)

- unblinded analysis of the primary efficacy endpoint ("progression to level >4 of modified WHO ordinal COVID-19 scale by Day 14" as a binary endpoint)
- calculation of conditional power (CP) given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study
    - if CP < 4%: recommend early stop for futility
    - if one-sided p-value < 0.0005: recommend early stop for superior efficacy
    - if 50% < CP < 80% and one-sided p-value ≥ 0.0005:
        - continue the study with an increased total sample size N*, so that CP with a total of N* subjects is increased to 80%, same value as the (unconditional) desired power in the study design
        - N*, however, is limited to 600 (which is twice the original sample size 300)
    - if 4% ≤ CP ≤ 50%: continue the study without a change in sample size (otherwise, the sample size would need to be increased too much or the maximum sample size of 600 would not be sufficient to come close to a CP of 80%.

The table on the next page illustrated the proposed procedure for a number of possible results observed at the 2nd interim analysis.

Table with examples for the adaptive sample size procedure described above using the Cui/Hung/Wang (CHW) approach using a weighted (fixed weights determined by the stage-wise sample sizes planned at the design-stage) test for the final analysis:

2nd IA results with n=150 subjects (50 for SOC, 100 for RESP301+SOC		**CP under observed trend for planned 300**	**Total sample size required for CP 80% under observed trend**	**CP under observed trend if total sample size capped by 600**
15 (30%)	5 (5%)	$p < 0.0005$ → early stop for superior efficacy		
15 (30%)	10 (10%)	99%	Not applicable	Not applicable
15 (30%)	15 (15%)	90%	Not applicable	Not applicable
15 (30%)	18 (18%)	66%	393	Not applicable
15 (30%)	19 (19%)	55%	483	Not applicable
15 (30%)	21 (21%)	34%	[798]	[67%]
15 (30%)	26 (26%)	3.99%	Early stop for futility	
12 (24%)	10 (10%)	92%	Not applicable	Not applicable
12 (24%)	12 (12%)	77%	321	Not applicable
12 (24%)	14 (14%)	54%	498	Not applicable
12 (24%)	15 (15%)	42%	[646]	[77%]
12 (24%)	18 (18%)	14%	[1800]	[31%]
12 (24%)	20 (20%)	2.6%	Early stop for futility	
10 (20%)	8 (8%)	85%	Not applicable	Not applicable
10 (20%)	10 (10%)	64%	412	Not applicable
10 (20%)	11 (11%)	50.4%	534	Not applicable

10 (20%)	13 (13%)	26%	[1014]	[55%]
10 (20%)	15 (15%)	10%	[2460]	[23%]
10 (20%)	18 (18%)	1.4%	Early stop for futility	

Quantities in [ ] indicate theoretical values as sample size would not be increased per the currently proposed adaptive design.

10.5.4 Overall Adaptive Design Performance

Finally, the Cui/Hung/Wang (CHW) approach is compared to the Chen/DeMets/Lan (CDL) approach without pre-defined fixed weights for the final analysis (which is valid as sample size may only be increased if CP (obtained under observed trend) at the second interim analysis exceeds 50%).

All the results below are for the group-sequential design with adaptive sample size re-estimation procedure as described in previous sections and based on 10.000 simulated trials each.

Scenario 1: true progression probabilities are 30% (SOC) and 15% (RESP301+SOC)

Scenario 2: true progression probabilities are 24% (SOC) and 14% (RESP301+SOC)

Scenario 3: true progression probabilities are 30% (SOC) and 10% (RESP301+SOC)

Scenario 4: true progression probabilities are 24% (SOC) and 20% (RESP301+SOC)

10.6 Appendix 6: Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CBC	complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CP	Conditional power
CRF	case report form(s) (paper or electronic as appropriate for this study)
CRO	contract research organization
CV	cardiovascular
DMC	Data Monitoring Committee
DRE	disease-related events
ECG	electrocardiogram
ECMO	Extra corporeal membrane oxygenation
EDC	electronic data capture
EoS	End of study
FDA	Food and Drug Administration
FiO_2	fraction of inspired oxygen
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IVRS	interactive voice response system

IWRS	interactive web response system
LFT	Liver function test
mHb	Methemoglobin
NEWS	National Early Warning Score
NHS	National Health Service
NO	Nitric oxide
NO_2	Nitrogen dioxide
NOx	Oxide of nitrogen
RT-PCR	Reverse transcriptase polymerase chain reaction
PP	Per protocol
PT	Prothrombin time
QTcF	QT interval corrected using Fridericia's formula
SAE	serious adverse event
SAP	statistical analysis plan
SD	Standard deviation
SoA	schedule of activities
SOC	Standard of care
SP	Safety population
SpO_2	Oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
TID	Three times daily
ULN	Upper limit of normal
US	United States
WHO	World Health Organization

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Investigator Agreement Page

Declaration of the Principal or Global Coordinating Investigator

Title: An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen (NOCoV2)

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the *Declaration of Helsinki* and the guidelines on Good Clinical Practice.

Principal or Global Coordinating Investigator

PPD

Title Page

Protocol Title:	**An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen (NOCoV2)**
Short Title:	An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301+SOC vs SOC in hospitalized participants with COVID-19 requiring supplemental oxygen
Compound:	RESP301
Indication:	Mild to moderate COVID-19
Study Sponsor:	Thirty Respiratory Limited PPD London, W1K 6PL, United Kingdom
Protocol Number.:	RESP301-002
Study Phase:	Phase 2/Phase 3
Regulatory Agency Identifying Number:	IND No: Pending EudraCT No: 2020-002120-37
Approval Date of Current Version:	Final, 10 Jun 2020; Amendment 2 (Version 3.0)
Date and Version of Previous Protocol:	05 Jun 2020; Amendment 1 (Version 2.0) 01 May 2020; Version: 1.0

Sponsor Signatory:

PPD

PPD

10th June 2020

Date

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Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY		
Document	**Version**	**Date**
Amendment 2.0	Version 3.0	10-Jun-2020
Amendment 1.0	Version 2.0	05-Jun-2020
Original Protocol	Version 1.0	01-May-2020

Amendment 2.0 (10-Jun-2020)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the EU.

Overall Rationale for the Amendment:

The protocol was updated to clarify that staggered dosing is required for all participants treated before completion of the first Independent Data Monittoring Committee (IDMC) review, and to clarify exclusionary criteria and monitoring of methemoglobin (mHb). Information was added to ensure that investigators are aware of the potential risk of interaction between nitric oxide (NO) and other NO donor agents. Other changes, with brief rationale, are summarized in the following table.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 Schedule of Activities (SoA)	Footnote 4 updated to include mHb assessment at the same time points as hematology and clinical chemistry assessments.	For monitoring and assessment of mHb against the discontinuation criteria.
Section 2.2.1 Risk Assessment Section 5.3 Exclusion criteria	Table 2-1 updated to include exclusionary mHb >1%. Exclusion criterion 13 updated to specify that mHb >1% is exclusionary.	The normal reference range for mHb is 0-1% (Longo et al, 2011).

Section # and Name	Description of Change	Brief Rationale
Section 4.1 Overall Design Section 6.1 Study Intervention Administered Section 9.6 Data Monitoring Committee (DMC)	Due to the urgency of the pandemic, if participants become eligible for the study during the IDMC review of the first 10 participants then they may be recruited. However, their administration of RESP301 will be staggered in the same way as for the first 10 participants. Once the IDMC review has been completed, and provided there are no safety concerns, further recruitment will continue as per protocol. The Sponsor and contract research organization (CRO) confirm that the report from the IDMC will be sent in a timely manner to all participating sites and investigators.	The staggered dosing approach will be used for all participants enrolled up to completion of the first IDMC safety review, to ensure appropriate safety procedures are in place for the first participants recruited into the study.
Section 6.5.1 Potential Drug Interactions	A new section was added to describe NO donor agents that may increase the risk of developing methemoglobinemia.	To ensure that investigators are aware of the potential risk of interaction between NO and other NO donor agents.
Section 7.1 Permanent Discontinuation of Study Intervention	Addition of Hy's law discontinuation criteria to the first bullet.	To ensure consistency with Appendix 10.3.
Whole document	Minor language and format changes.	For improved clarity and readability.

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1 Protocol Summary

1.1 Synopsis

Protocol Title: An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen (NOCoV2)

Sponsor Protocol No.: RESP301-002

Study Phase: Phase 2/Phase 3

Sponsor: Thirty Respiratory Limited

Rationale:

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design. Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer 3 times a day (TID) for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 4 in the modified World Health Organization (WHO) ordinal scale into levels >4	• Proportion of participants who progress to level >4 of modified WHO ordinal scale due to COVID-19 by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO2	• Change in room air SpO2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time

Objectives	Endpoints
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement to a lower level (<4) of modified WHO ordinal scale • Time to progression to a higher level (>4) of modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	

• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements
	• Physical examinations
	• Vital signs
	• Concomitant medications
	• Counts and cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

Overall Design:

- Open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 added to standard of care (SOC) in hospitalized patients with COVID-19 requiring supplemental oxygen.

- Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as detailed in Section 6.3.

- The study treatment will be permanently discontinued prior to 10 days if the participant:

    - Improves to level 1 or 2 of the modified WHO ordinal scale;
    - Progresses to a level > 4 of the modified WHO ordinal scale;
    - Experiences an event that requires permanent discontinuation as described in Section 7.1.

- An Independent Data Monitoring Committee (IDMC) will be responsible for closely reviewing the safety and efficacy data from interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

- A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). Each RESP301 dose will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6).

Disclosure Statement: This is a parallel group treatment study with two arms that are open-label.

Number of Participants:

Approximately 300 adult (male or female) participants will be randomly assigned to study intervention (200 to the Investigational arm and 100 to the Control arm).

Intervention Groups and Duration:

The total study duration for a participant from screening to last follow up will be up to 30 days (±2 days). The study will be divided into the following periods:

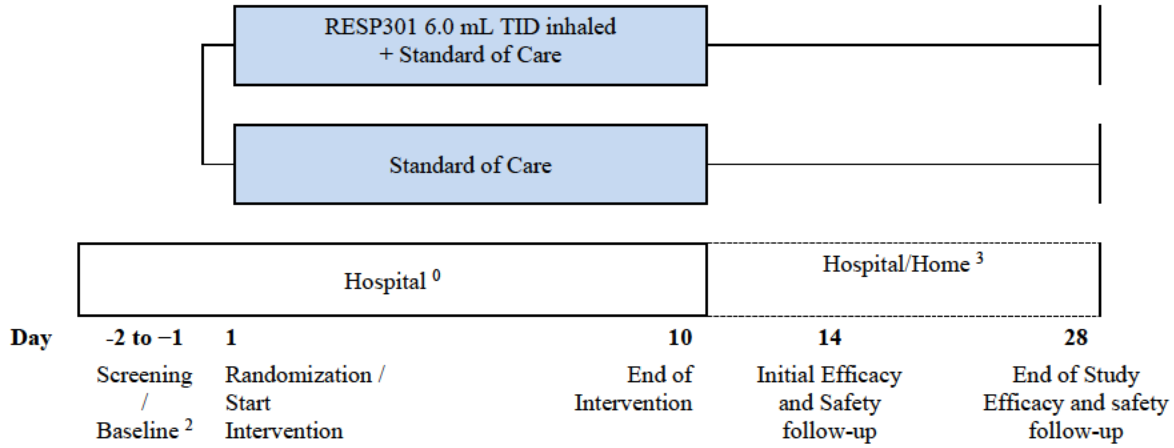
- Screening period: starts up to 2 days (48 hours) prior to and extends up to the day of treatment initiation (Day 1); Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28 (both ±2 days)
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±2 days)

Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer TID for up to 10 days in addition to the SOC, while participants in the Control arm will receive SOC alone.

Data Monitoring Committee: Yes

1.2 Schema

Figure 1–1: Study Design



TID=3 times daily

1 Participants may be discharged from hospital before Day 10.

2 Screening period may include Day 1, as participants may be screened and randomized on the same day provided all eligibility criteria are met and the participant had sufficient time to consider their participation in the study.

3 The post-treatment efficacy and safety follow-ups may be conducted by telephone for participants who are discharged from the hospital at the time.

1.3 Schedule of Activities (SoA)

Table 1-1 Schedule of Activities (SoA)

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Informed consent	X							Optional tokenization ICF to be provided for a subset of participants in the United States (see Section 8.4)
Confirmation of COVID-19 infection by nasopharyngeal RT-PCR [3]	X							
Inclusion and exclusion criteria	X	X						Recheck clinical status before 1st dose of study intervention
Demographics	X							
Physical examination including height and weight	X			X	X		X [2]	Full physical exam and height only at screening

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Pregnancy test (urine)	X							All women, including women of childbearing potential and pregnant women, may be enrolled in the study. Pregnancy status should be recorded (see Section 8.3.5). Note: Only females of child bearing potential and pregnant females need to undergo a screening pregnancy test.
Medical history (includes substance usage)	X							History of substance usage or abuse to be recorded but is not exclusionary
Medication history	X							In the prior 3 months
Laboratory assessments (including liver chemistries) [4]	X [4]	X [4]	X (Days 3 and 7 only) [4]	X [4]	X [4]		X [2,4]	
12-lead ECG	X							After resting supine for approximately 5 minutes. QRS, QT, QTcF, PR, RR, rate
Vital signs [5]	X	X	X [5]	X	X		X	Within 15 minutes prior to study intervention administration

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Randomization		X						
Study intervention, TID [6]		X	X	X				A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). Each RESP301 dose will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6). Where possible study intervention should be administered at approximately the same times each day At least 6 hours between 2 consecutive doses
AE/SAE review	X	X	X	X	X	X	X	
Concomitant medication review	X	X	X	X	X	X	X	
Oxygen / respiratory assessment [7]	X	X	X	X				
SpO_2 on room air (after at least 1 minute) prior to study intervention administration and immediately at the end of nebulization [6]		X	X	X				See Section 8.1.2

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Modified WHO ordinal scale	X	X	X	X		X	X	See Section 8.1.1
NEWS 2 assessment [7]		X	X	X				Prior to study intervention in the morning. See Section 8.1.3

Abbreviations: AE=adverse event; aPTT= activated partial thromboplastin time; CBC=complete blood count; Chem 7=blood chemistry panel (sodium, potassium, chloride, bicarbonate/CO_2, blood urea nitrogen, creatinine, glucose); CV=cardiovascular; ECG=electrocardiogram; IDMC=Independent Data Monitoring Committee; LFTs=liver function tests; mHb=methemoglobin; PT/INR=prothrombin time/international normalized ratio; RT-PCR=reverse transcription-polymerase chain reaction; SAE=serious adverse event; TID=three times daily.

1. Screening and randomization may be on the same day, providing all eligibility criteria are met.
2. For participants who are already discharged at either the Day 14 or Day 28 (EoS) follow-up, there will be a phone call to check AEs/SAEs, concomitant medications, and modified WHO ordinal scale. At EoS, safety laboratory tests (nitrite and mHb only) and weight will be collected for participants in hospital only.
3. COVID-19 infection must be confirmed and documented in chart with a positive result on a validated test.
4. At Screening: CBC, chem 7, mHb, LFTs, and coagulation (PT/INR, aPTT).

   On Day 1, Day 3, Day 7 and Day 10, or early withdrawal: CBC, chem 7 (per SOC), and mHb.

   On Day 28: nitrite and mHb (participants in hospital at EoS only).
5. Vital signs: temperature, heart rate, blood pressure, respiratory rate. Vital signs to be repeated as per SOC during treatment period.
6. Study intervention comprises the following procedures which are to be done in order and as quickly as possible:
   a. Recording of oxygen flow level including mode (nasal cannula, mask)
   b. Recording of SpO_2 and heart rate
   c. Discontinuation of supplemental oxygen administration *
   d. Close participant oversight by trained health care professional until SpO_2 is stable on ambient air after one minute at least *
   e. Recording of SpO_2 * pre-nebulization on ambient air
   f. Preparation of the admixture of RESP301*
   g. Administration of the nebulization via a vibrating mesh nebulizer while monitoring SpO_2 and heart rate (alarms set to 89% and 120 beats / min, or as indicated by the Investigator, for stopping study intervention administration and resuming supplemental oxygen) * If the participant is unable to tolerate nebulization without supplemental oxygen, nasal oxygen may be administered during nebulization.
   h. Discontinuation of nebulization after 5 minutes or no product left in the device, whichever comes first *
   i. Recording of SpO_2 * post nebulization on ambient air
   j. Resuming supplemental oxygen *
   k. Participant oversight until SpO_2 is stable *
   l. Recording of SpO_2 *

   * *for participants on active treatment arm only*
7. Immediately before participant is discontinued from oxygen for study intervention administration (*for participants on active treatment arm only*) and assessment of SpO_2 at room air.

2 Introduction

2.1 Background

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by a novel coronavirus (SARS-CoV-2). COVID-19 was first described in Wuhan, China, in December 2019 and is now a global pandemic (Matos et al, 2020). Most of those affected have milder illness (80%), 15% will be severely ill (require oxygen) and 5% will require intensive care unit care (Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Of those who are critically ill, most require early intubation and mechanical ventilation. Other complications include septic shock and multi-organ failure, including acute kidney injury and cardiac injury (Yang et al, 2020). Older age and comorbid diseases, such as chronic obstructive pulmonary disease (COPD), hypertension, and diabetes, increase risk of death (Huang et al, 2020; Zhou et al, 2020). The virus is highly contagious and spread via respiratory droplets, direct contact and, if aerosolized, airborne routes. The most common symptoms include fever, fatigue, dry cough, and shortness of breath.

A critical early component of innate host defense in the airway is the ability of respiratory epithelial cells to produce high levels of NO (Kao et al, 2001). NO functions as a signaling molecule in initiation of the inflammatory response to viruses, and also has direct antiviral effects (Folkerts et al, 1998). The airway epithelium has highly efficient nitric oxide (NO) synthetic machinery which is amplified in viral infection. Healthy human airway epithelium has abundant expression of the endothelial enzyme NOS II due to continuous transcriptional activation of the gene in vivo. Loss of NO synthesis in lung diseases predisposes individuals to increased virus/microbe infection (Xu et al, 2006).

The physiological roles of NO and the enzymatic pathways for its synthesis via NO synthase have been clearly established for many years (Tucker et al, 2007). In particular, NO has been demonstrated to have potent anti-microbial properties against a wide range of pathogens. An alternative non-enzymatic synthetic pathway for NO synthesis has been developed, which generates NO and related higher oxides of nitrogen (NOx) via the chemical reactions of acidified nitrite (Hardwick et al, 2001, Tucker et al, 1999). A solution of co-mixed NO/NOx and ascorbic acid (RespiNOS), delivered by nebulizer, was found to be safe and well tolerated in healthy volunteers (Tucker et al, 2007). Spectral investigations further confirmed that there were no potentially harmful moieties present in the solution. The study concluded that RespiNOS had potential for use as a broad-spectrum anti-microbial agent in patients with chronic bronchial sepsis such as bronchiectasis, COPD, and cystic fibrosis.

RESP301 is a NO-generating liquid designed to release NO in situ in the upper airways and deep in the alveolar spaces. RESP301 is an admixture solution of two precursor solutions mixed together at point of care for immediate inhaled administration via a CE marked handheld nebulizer and has specific advantages (see below) over inhaled NO gas in treating patients with COVID-19 during the current pandemic. It has shown high in vitro activity against respiratory pathogens, both viral and bacterial.

Key advantages of RESP301 over inhaled NO gas are:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

The Sponsor has conducted a number of supportive in vitro studies to assess the activity of NO-generating solutions against major respiratory viruses responsible for infections in humans. These studies include the following:

- In vitro studies to demonstrate the effects of RESP301 on SARS-CoV-2 replication
- In vitro study of RESP301 against influenza A (H1N1) and human rhinovirus
- Determination of the anti-viral efficacy of NO-releasing formulations against two strains of virus (influenza A virus [H1N1] and human rhinovirus 16)
- Anti-microbial activity of acidified nitrite solutions against intracellular drug sensitive and drug resistant *Mycobacterium tuberculosis* and *M. abscessus* infections
- Anti-microbial activity of RESP301 when used alone and in combination with antibiotics

Recent data from experiments at two independent laboratories using two different isolates of SARS-CoV-2 suggest that incubation with RESP301 for 36 to 48 hours reduced viral load to levels below the limit of detection of the assay at concentrations which were not associated with cytotoxicity. The limiting factor of the experiments was the slow growth rate of the viral controls; reduction in viral load was 2.2 and 4 Log_{10} median tissue culture infectious dose ($TCID_{50}$).

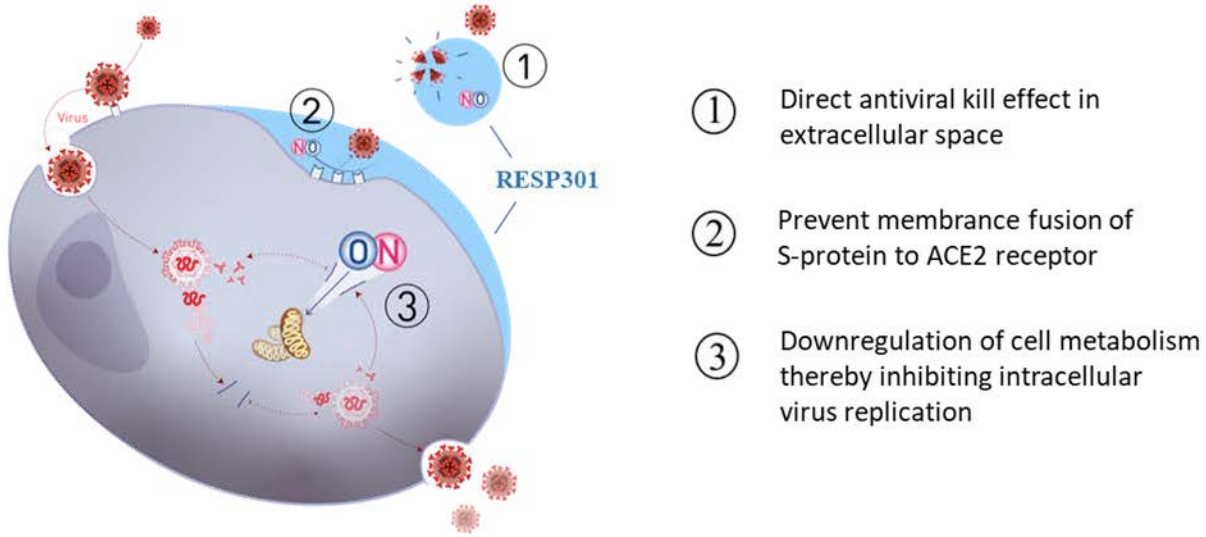
Studies of inhaled nebulized sodium nitrite (AIR001) in healthy subjects and in patients showed that inhaled acidified nitrite produces dose proportional plasma pharmacokinetics without accumulation following repeated administration, with low systemic blood levels of nitrite and

methemoglobin (<3%) (Rix et al, 2015). Inhaled nitrite (AIR001) at doses up to 90 mg three times daily (TID) displayed a good safety profile and is well tolerated (Parsley et al, 2013; Simon et al, 2016), thus supporting the investigation of RESP301 in the clinical setting.

RESP301 delivers NO in a specifically focused way and with a unique mode of administration. It is an important evolutionary step in delivering NO that is more physiological and overcomes the technical difficulties and efficacy limitations of current NO gas therapy. Whereas inhaled NO gas has been used to treat patients with virus-induced acute respiratory distress syndrome and is being tested currently in COVID-19 patients, it focuses on increasing vascular permeability and is not sufficiently targeted at the infection, it is rapidly dispelled with exhalation and is oxidized in air to toxic NO_2. By comparison, RESP301 generates NO in a liquid environment that comes in direct contact with the virus and the virus-infected cells. As a result, RESP301 has a better targeted approach, and lower concentrations of NO are required, with a much more portable delivery system. An important environmental safety factor is that RESP301 produces negligible levels of NO_2 generated and exhaled NO.

In generating NO, RESP301 has at least three distinct mechanisms of action in fighting virus infection, two of which are common to many viruses and one is very specific to coronaviruses, such as SARS-CoV-2 (Figure 2–1).

Figure 2–1: Mode of Action of RESP301 Against SARS-Cov-2



1. Nitric oxide has a direct kill effect on the virus in the extracellular space. Nitric oxide-mediated nitrosylation of viral and host macromolecules appears to block viral replication and this has been demonstrated for several viruses (Saura et al, 1999; Basu et al, 1999). Enzymes, such as proteases (reverse transcriptases, and ribonucleotide reductase), vital for the life-cycle of the virus, are targets for NO nitrosylation (Benz et al, 2002; Colosanti et al, 1999).
2. In coronaviruses like SARS-CoV-2, NO specifically prevents membrane fusion of the S-protein (the spike protein that gives the virus its characteristic crown-like appearance) to the angiotensin-converting enzyme 2 receptor on the host cell.
3. Nitric oxide can attack the virus indirectly. Since viruses are non-living entities and do not have their own metabolism, they hijack the host cell's metabolic processes, and derive their energy and specific cellular substrates from the host cell. Nitric oxide suppresses the virus-induced hyperactivity in the host cell and thereby deprives the virus of its crucial supply chain.

RESP301 efficiently delivers with all three modes of action and is therefore considered an ideal candidate for clinical trials in COVID-19.

A detailed description of the chemistry, pharmacology, efficacy, and safety of RESP301 is provided in the Investigator's Brochure.

2.2 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of RESP301 may be found in the Investigator's Brochure.

2.2.1 Risk Assessment

Table 2-1 Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention RESP301		
Participants may need to be discontinued from supplemental oxygen for	Study treatment is to be administered via a vibrating mesh nebulizer which is designed for oral inhalation.	Participants unable to be safely discontinued from supplemental oxygen will be

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
several minutes to receive nebulized study treatment		excluded (Exclusion criterion 2) Treatment administration is to be performed as quickly as possible (Section 1.3) Oxygen saturation (SpO_2) and heart rate will be monitored throughout the study intervention administration (Section 1.3) In case of decrease in SpO_2 or clinical signs of intolerance to nebulization, RESP301 treatment may be paused temporarily and supplemental O_2 resumed or the study treatment may be discontinued permanently (see Section 7.1) per Investigator assessment.
Risk of methemoglobinemia	Very low levels are produced but as methemoglobinemia has been reported with continuous NO gas administration, the risk cannot be firmly ruled out	Participants with a history of methemoglobinemia will be excluded (Exclusion criterion 4), methemoglobin (mHb) level >1% is exclusionary (exclusion criterion 13), and mHb is included in safety laboratory tests (Section 1.3)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Risk of clinical bronchial hyper-responsiveness related to nebulization	Excipients in the nebulized RESP301 include mannitol which is a known bronchial irritant at the average dose of a positive mannitol test (239.0 ± 185.0 mg, Brannan et al, 2005). While the dose used in this study (54.66 mg) is lower than the average dose for a positive mannitol test, it has not yet been tested in COVID-19 patients and may potentially cause or exacerbate cough or bronchospasm in susceptible individuals in this specific population	Participants with a known history of moderate or severe bronchial hyperreactivity (such as in asthma) or presence of signs of significant bronchospasm on examination will be excluded from study participation (Exclusion criterion 5) For the first 10 participants treated before the first Independent Data Monitoring Committee (IDMC) safety review (Section 9.6), RESP301 will be administered by intermittent inhalation until the intended dose is delivered (Section 6.1), so that the treatment can be quickly adjusted and rescue bronchodilator initiated if needed (Section 6.5.2) After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by continuous inhalation.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Nebulization	The delivered dose of RESP301, estimated to be ~40% of the masses listed in Table 6-2, can be affected by patient technique and inspiratory capacity leading to inter- and intra-patient variation in actual doses of these ingredients, given the acute nature and high disease burden in this proposed clinical setting	Training will be provided to study staff to minimize variation in dose
Other		
Risk of spreading SARS-CoV-2	SARS-CoV-2 is known to be transmissable via respiratory droplets and any interventions that may potentially increase cough or aerosolization of respiratory secretions of an infected patient may increase risk of spread of disease	Proper personal protective equipment including appropriate mask, face shield, gown and gloves should be used at all times. When possible, the site staff should not remain in the room during study intervention administration (providing that continuous O_2 monitoring can be conducted remotely and study intervention can be administered correctly)

2.2.2 Benefit Assessment

To date, no treatment of COVID-19 has demonstrated clinical efficacy. Patients and their physicians are critically in need for treatments that decrease the risk of severe levels of disease,

particularly the rate of intubation or other ventilatory support as they significantly lead to fatal outcomes. Considering the current pandemic, even a limited improvement in the rate of progression to severe stage of the disease would provide sizable benefits for patients and society.

Nitric Oxide is already marketed (e.g., INOmax®, NOXIVENT™, etc) in the United States (US) and other countries as a gas for continuous use in preterm and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension in conjunction with ventilatory support and other appropriate agents. It is well tolerated, although under continuous use, a small number of cases of methemoglobinemia have been reported (NOXIVENT™ FDA Prescribing Information).

Nitric oxide has been shown in vitro to inhibit the replication cycle of severe acute respiratory syndrome coronavirus (Akerström S et al, 2005). In addition, NO is a naturally occurring and potent antimicrobial agent in the human body, which is active against viruses, bacteria, fungus, and yeasts (Fang, 1997). In particular, NO inhibits replication in vitro in a number of respiratory viruses including influenza A and B (Rimmelzwaan et al, 1999, Regev-Shoshani et al, 2013), human rhinovirus (Sanders et al, 1998), and respiratory syncytial virus (Ali-Ahmad D et al, 2003).

The ability of RESP301 to have a marked antibacterial action is highly relevant in the context of treating patients with SARS-CoV-2 infection since superimposed bacterial infection is a critical factor and a major cause of morbidity and mortality. RESP301 would restore and replenish the NO deficiency in patients who have succumbed to SARS-CoV-2 infection. RESP301 also has key advantages over inhaled NO gas, as discussed in Section 2.1.

A vibrating mesh nebulizer is advised for administration of RESP301. Amongst the suitable nebulizers, the device chosen for the study is a commercially available, CE-marked vibrating mesh device that delivers a mist of fine droplets in the size range required for pulmonary deposition (Mass Median Aerodynamic Diameter < 5 μm). This has been shown to be ideally suited for penetration deep into the alveolar spaces of the lung. As with all nebulizers in clinical use, there is a residual amount of drug that remains in the nebulizer so that not the full amount of drug is administered. The device operates continuously once initiated and automatically switch off once the medication has been delivered. The device may be used in pediatric and adult populations, as permitted by the prescribed medication, and is suitable for use in home environments or hospital/clinic settings.

As testing inefficient product would distract sites from participating in other research efforts, two interim analysis for futility purpose have been planned.

2.2.3 Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to participants in this first-in-man study, the potential risks identified in association with RESP301 are justified by the anticipated benefits that may be afforded to participants with COVID-19.

3 Objectives and Endpoints

Table 3-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 4 in the modified World Health Organization (WHO) ordinal scale into levels >4	• Proportion of participants who progress to level >4 of modified WHO ordinal scale due to COVID-19 by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO_2	• Change in room air SpO_2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement to a lower level (<4) of modified WHO ordinal scale • Time to progression to a higher level (>4) of modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	
• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements • Physical examinations • Vital signs

Objectives	Endpoints

	• Concomitant medications
	• Counts and cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

4 Study Design

4.1 Overall Design

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study in hospitalized participants with COVID-19 requiring supplemental oxygen (WHO ordinary scale level 4) using a sequential adaptive design to evaluate the efficacy and safety of RESP301 added to standard of care (SOC). Approximately 300 adult (male and female) participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a vibrating mesh nebulizer TID for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

The study will be divided into the following periods:

- Screening period: starts up to 2 days (48 hours) prior to and extends up to the day of treatment initiation (Day 1); Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10

- Efficacy follow-up: Day 14 and Day 28 (±2 days)
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±2 days)

After screening, eligible participants will be randomized to either RESP301+SOC or SOC alone on Day 1, and the study treatment will be initiated as applicable (Table 1-1). Screening and randomization may happen on the same day (Day 1).

An individual administration of study intervention will be stopped, and supplemental oxygen resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Section 4.2 and Section 8.1.2). However, the participant may continue with subsequent doses if the Investigator judges that the benefit / risk ratio remains positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored. Inhalation of study intervention should be completed within 20 minutes. A staggered dosing approach will be used for the first 10 participants (and enrolment up to completion of the first IDMC safety review) to monitor tolerability and response to RESP301 (see Sections 6.1 and 9.6).

The study treatment will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale;
- Experiences an event that requires permanent discontinuation as described in Section 7.1.

In the above cases, participants will be considered as ongoing in the study until the final Safety follow-up phone call (Day 28). Participants will be withdrawn from the study prior to Day 10 (Early withdrawal) only if they withdraw consent.

The total study duration for a participant from screening to last follow up will be up to 30 days (±2 days).

Participants will be stratified by country and presence of risk factor(s) for severe outcomes of COVID-19, based on comorbidities and age, as detailed in Section 6.3.

Two interim analyses are planned (see Section 9.5 for details).

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

4.2 Scientific Rationale for Study Design

NO is a naturally occurring and potent antimicrobial agent in the human body which has been shown in vitro to inhibit replication of severe acute respiratory syndrome coronavirus (Akerström S et al, 2005), including SARS-CoV2, and other respiratory viruses. NO as an inhaled gas is already marketed in the US and other countries (see Section 2.2.2) but has disadvantages over NO produced locally in the oropharynx or lung airways (lengthy treatment requiring NO canisters, the inhaled NO is expelled in exhalation, the NO gas oxidizes in air to form toxic NO_2 which is a potential lung irritant and a contaminant in the patient's environment, and the half-life of NO in air is short).

RESP301 has potential advantages over inhaled NO gas:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

In this study, the effect of RESP301 as an add on treatment to SOC will be evaluated for its efficacy in reducing rate of progression to a more severe level of COVID-19 and for safety by comparison with SOC alone in hospitalized COVID-19 patients. A sequential adaptive design was chosen in order to assess futility and sample size after interim analyses (see Section 9.5). The study design was developed in accordance with the latest WHO and regional regulatory agencies guidelines.

Several risks factors for severe or fatal outcomes have been reported (Bialek et al, 2020; Huang et al, 2020; Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020; Rong-Hui et al, 2020; Zhou et al, 2020). To minimize bias, the results will be stratified according to the known risk factors as described in Section 6.3.

In this study, measures will be in place to trigger discontinuation of study intervention at a SpO_2 limit pre-agreed at the site (see Section 8.1.2). There is no critical level of oxygen saturation below which tissue hypoxia occurs due to the large number of variables that contribute to hypoxia at the tissue and cellular level (temperature, pH, tissue blood flow). As a result, there is no consensus about what constitutes normal and abnormal oximetry (ATS/ACCP, 2003). To support and guide the team in charge of study intervention administration, a default value of

SpO_2=89% and heart rate (120 beats/min) has been proposed as a suitable limit of tolerance. However, for the reason stated above, this limit can be overruled by the Investigator in either direction.

Newly released guidelines for ongoing clinical trials during the COVID-19 pandemic have emphasized the need to reduce the burden on sites and clinical trial personnel/investigators whether they be administrative, site visit, or other burdens (ACRO, 2020; EMA, 2020; HRS, 2020). Thus, without blinding and a placebo, along with minimal procedures, the additional burden on clinical staff has been reduced as much as possible.

4.3 Justification for Dose

Studies with nebulized sodium nitrite (AIR001) in healthy subjects and in patients have shown that inhaled nitrite produces dose proportional plasma pharmacokinetic without accumulation following repeated administration, with low systemic blood levels of nitrite and methemoglobin (<3%). Inhaled nitrite at doses up to 90 mg TID displayed a good safety profile and is well tolerated (Rix et al, 2015; Parsley et al, 2013; Simon et al, 2016).

In this study, 6.0 mL RESP301 (delivered dose 62 mg) will be administered via a vibrating mesh nebulizer, TID with at least 6 hours between each dose. Each 6.0 mL dose for nebulization contains the masses described in Table 6-2.

4.4 End of Study Definition

A participant is considered to have completed the study if he/she has completed the Treatment Period and the Follow-up Period (through the Day 28 EoS Follow-up). End of the study is defined as the last participant's last visit or follow up call.

5 Study Population

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

The study population will consist of male and female COVID-19 patients, including pregnant women and women of child bearing potential. Participants must be able to provide written consent and meet all the inclusion criteria and none of the exclusion criteria.

5.1 Study Rationale

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 plus SOC versus

SOC alone in hospitalized patients with COVID-19 requiring supplemental oxygen (WHO level 4).

Each constituent (see Section 6.2) of RESP301 is used as SOC for various conditions. Product application is straightforward, requiring a few minutes of inhalation using a standard nebulizer. RESP301 is likely to be beneficial in treating patients with COVID-19 who are not receiving ventilation but are using supplementary oxygen in order to maintain a safe level of SpO_2.

Considering the well-established and global use of NO and the device, and the current public health emergency resulting from the COVID-19 pandemic, a Phase2/3 study of RESP301 is considered reasonable in order to generate efficacy data in patients infected with COVID-19.

5.2 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant is ≥18 years of age, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participant has laboratory-confirmed SARS-CoV-2 infection as determined by reverse transcriptase polymerase chain reaction (RT-PCR) or other approved clinical testing prior to randomization.
3. Participant is hospitalized in relation to COVID-19, requiring supplemental oxygen to maintain SpO_2 at a safe level (WHO level 4).

Sex

4. Participant is male or female. All females of childbearing potential, including pregnant females, must consent to urine pregnancy testing at screening to be eligible for the study. (Females who are not of childbearing potential do not need to undergo a pregnancy test at screening).

Informed Consent

5. Participant is capable of giving signed informed consent as described in Appendix 1 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.3 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Rapidly deteriorating or likely to require escalation to high flow oxygen, invasive or non-invasive ventilatory support within 24 hours according to Investigator's opinion.
2. Unable to safely receive a nebulized treatment for approximately 4 minutes according to Investigator's opinion.
3. Unable to receive or considered ineligible for invasive or non-invasive ventilatory support.
4. History of methemoglobinemia.
5. Uncontrolled asthma or history of severe bronchospasm.
6. Severe (requiring baseline oxygen therapy > 12 h/day prehospitalization) chronic respiratory disease (e.g., known COPD, pulmonary arterial hypertension, idiopathic pulmonary fibrosis, interstitial lung disease).
7. Suspected or confirmed untreated, active tuberculosis.
8. Severely immune-compromised participants in Investigator's opinion.
9. Recent (within 3 months) active coronary artery disease or decompensated heart failure (New York Heart Association class 3-4).
10. Presence of tracheostomy.

Prior/Concomitant Therapy

11. Chronic (≥4 weeks) use of corticosteroids >10 mg/day of prednisone or equivalent within 4 weeks of randomization.

Prior/Concurrent Clinical Study Experience

12. Participation in other clinical investigations utilizing investigational treatment or within 30 days / 5 half-lives whichever is longer.

Diagnostic Assessments

13. Clinically significant abnormalities in clinical chemistry or hematology at screening, defined as:

- Methemoglobin level >1% (Longo et al, 2011)
- Platelet count <50,000 mm^3
- Alanine aminotransferase or aspartate aminotransferase >5 × upper limit of normal (ULN).
- Estimated glomerular filtration rate <30 mL/min/1.73 m^2 (modification of diet in renal disease formula) or requiring hemofiltration or dialysis.

Other Exclusions

14. Anticipated transfer to another hospital which is not a study site during the treatment period.
15. Allergy to any of the components of the study intervention.

5.4 Lifestyle Considerations

No restrictions are required.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography and reason for screen failure (e.g., eligibility requirements failed).

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

5.5.1 Screening and Enrollment Log and Participant Identification Numbers

The participant's enrollment will be recorded in the Screening and Enrollment Log.

Upon enrollment, each participant will receive a unique participant identification number. Participant numbers must not be re-used for different participants.

6 Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1 Study Intervention Administered

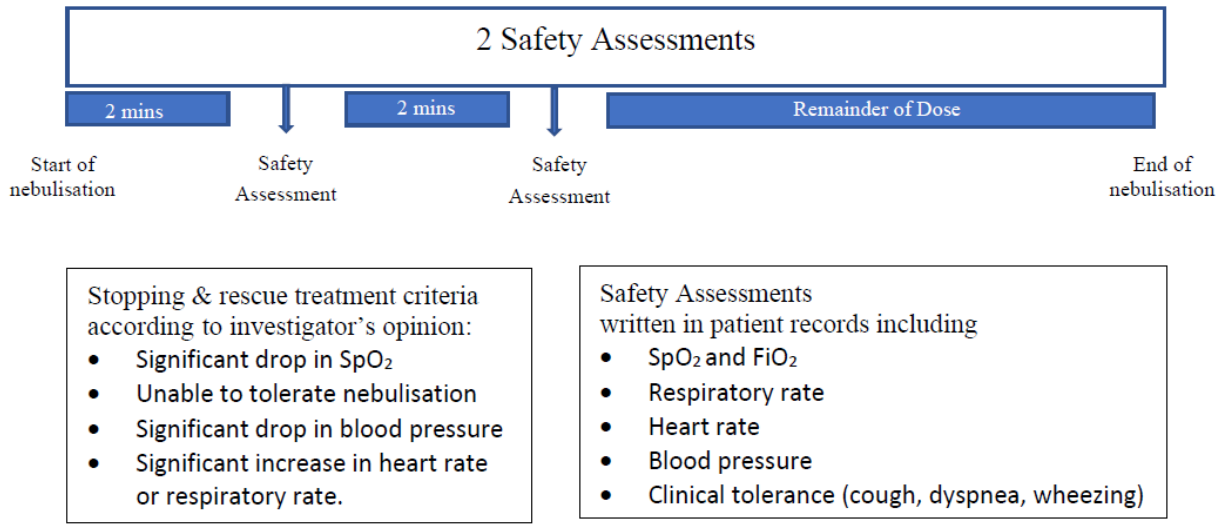
Table 6-1 Study Intervention(s) Administered

Study Treatment	RESP301 + SOC
Intervention Name:	RESP301
Type:	Drug
Dosage Formulation:	Admixture for inhalation
Unit Dose Strength(s):	Delivered dose (62 mg) sodium nitrite ($NaNO_2$) (6 mL of NO-generating admixture of 150 mM $NaNO_2$, 50 mM mannitol and 100 mM citric acid)
Dosage Level(s):	One 6 mL-inhalation TID (every 8 hours) with at least 6 hours between 2 consecutive doses.
Route of Administration:	Inhalation
IMP and NIMP:	IMP
Sourcing:	RESP301 provided centrally by the Sponsor.
Dosing Instructions:	A vibrating mesh nebulizer will be used to administer the RESP301, under the direction of the study physician. Treatment is to commence within 5 minutes of RESP301 preparation. For the first 10 participants (and enrolment up to completion of the first IDMC safety review) RESP301 will be administered by intermittent inhalation to monitor tolerability and response to RESP301. After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by a continuous inhalation. Before initiating each RESP301 inhalation, supplemental oxygen will be interrupted for a few minutes until SpO_2 levels are stable. Depending on the extent of SpO_2 decrease, the RESP301 inhalation may be initiated or supplemental oxygen may be resumed at the discretion of the investigator. After treatment initiation, in case of SpO_2 decrease, RESP301 inhalation may be paused and supplemental oxygen resumed. If the participant is unable to tolerate nebulization without supplemental oxygen, nasal oxygen may be administered during nebulization.
Packaging and Labeling:	Study intervention will be provided as two separate vials: $NaNO_2$/mannitol (3 mL) and citric acid buffered to pH 5.4 (3 mL), which will be labeled as required per country requirement.

Staggered dosing approach for the first 10 participants:

For the first 10 participants treated, and enrolment up to completion of the first IDMC safety review (Section 9.6), RESP301 dose will be administered in a staggered approach: after 2 minutes of inhalation, treatment is briefly paused to assess the participant. Provided there is no evidence of bronchospasm, treatment is continued for a further 2 minutes, after which treatment is again briefly paused for participant assessment. Thereafter, provided there is no evidence of bronchospasm and the participant is able to continue, the dose is completed (Figure 6-1), so that treatment can be quickly adjusted and rescue bronchodilator initiated if needed (Section 6.5.2). After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by a continuous inhalation (see also Section 9.6). Due to the urgency of the pandemic, if participants become eligible for the study during the IDMC review of the first 10 participants then they may be recruited; however, their administration of RESP301 will be staggered in the same way as for the first 10 participants.

Figure 6-1: Staggered Dosing Approach for the First 10 Participants



6.1.1 Medical Devices

1. Medical device (not manufactured by or for Thirty Respiratory Limited) provided for use in this study will be an FDA-approved and CE marked vibrating mesh nebulizer.
2. Instructions for medical device use will be provided in the User Manual.

6.1.2 Device Training

Training will be provided to ensure all study staff are familiar with the device, including mixing the solutions, adding the admixture to the device and cleaning the device.

6.2 Preparation, Handling, Storage, and Accountability

6.2.1 Preparation of Study Intervention Product

RESP301 is prepared at bedside by mixing two sterile 3 mL solutions (one of $NaNO_2$/mannitol and one of citric acid buffered to pH 5.4). The solution is to be nebulized immediately or within 5 minutes post mixing as per the instructions given in the SoA (Table 1-1) and, for the first 10 participants, as in Section 6.1 above (staggered dosing). The first administration of study intervention should be provided under medical supervision.

RESP301 is mixed in the nebulizer chamber before use following the steps below (see Table 6-2 for details of composition):

1. First the vial marked A ($NaNO_2$ and mannitol) is opened and its contents are poured into the nebulizer chamber.
2. Next, the vial marked B (citric acid) is opened and its contents are poured into the nebulizer chamber.
3. The nebulizer lid should be closed, and the nebulizer should be swirled gently for a few seconds to mix the two solutions. The nebulizer should not be inverted.

The inhalation of the medication should start within 5 minutes of mixing the solutions and the inhalation process should be complete within 20 minutes of starting of inhalation.

This combined mildly acidified nitrite solution (6 mL) is inhaled through the mouth using a vibrating mesh nebulizer under the direction of a physician. The purpose of the acidified nitrite aerosol is to deliver NO to the participant. The delivered dose of RESP301, assessed via simulated tidal breathing is estimated to be ~40% of the masses listed below; it should be noted that delivered dose can be further affected by participant's breathing pattern and inspiratory capacity and so inter- and intra-participant variation in actual doses of these ingredients may vary, given the acute nature and high disease burden in this proposed clinical setting.

Table 6-2 Composition of Drug Product, Diluent and Admixture

	Material	Function	Quantity per vial
Drug Product	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Diluent	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Acidified Drug Product Mixture (RESP301)	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**6.0 mL**

6.2.2 Storage and Accountability of Study Intervention

The Investigator or designee must document whether appropriate temperature conditions (<25°C) have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (e.g., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study intervention are provided in the Investigator Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an interactive voice response system (IVRS)/interactive web response system (IWRS). The site will contact the IVRS/IWRS prior to the start of study intervention

administration for each participant. The site will record the intervention assignment on the applicable case report form (CRF), if required.

Potential bias will be reduced using a central stratified randomization to assign participants into one of the study treatment arms, RESP301+SOC or SOC (control), with a randomization ratio of 2:1. Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as follows:

1. Age >65 years
2. Ongoing or currently treated diabetes mellitus
3. Ongoing or currently treated hypertension
4. Ongoing or currently treated cardiovascular disease
5. Ongoing or currently treated chronic lung disease
6. Cancer history of less than 3 years, basal cell skin carcinoma excluded
7. Ongoing or currently treated chronic kidney disease

Participants will be stratified as no risk factor (none of the above criteria), one risk factor (one single of the above) or high-risk factor (2 or more of the above).

6.4 Study Intervention Compliance

Participants will receive study intervention directly from the Investigator or designee, under medical supervision. The date and time of each inhalation will be recorded in the source documents and recorded in the CRF. The study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Concomitant Therapy

During the study, participants will receive institutional SOC for the treatment of COVID-19.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency and route

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1 Potential Drug Interactions

Investigators should be aware of the potential interaction between NO generated by RESP301 and that generated from NO donor agents, such as prilocaine, sodium nitroprusside, and nitroglycerine, that may increase the risk of developing methemoglobinemia.

6.5.2 Rescue Medication

For participants treated before completion of the first safety review by the IDMC (Section 9.6), at the time of RESP301 treatment, a ready-to-use nebulization of a bronchodilator (short acting beta agonist such as salbutamol/albuterol according to investigational site standard practice for acute bronchospasm) may be administered to participants in case of bronchospasm.

After reviewing safety data, the IDMC will decide whether the availability of rescue bronchodilator at the time of RESP301 treatment is still required.

Although the use of rescue medications is allowable for the first 10 participants, the use of rescue medication should be delayed, if possible, for at least 2 minutes following each administration of study intervention. The date and time of rescue medication administration as well as the name of the rescue medication must be recorded.

6.6 Intervention After the End of the Study

After the end of the study, participants may continue their SOC (if any). Study intervention for COVID-19 will not continue beyond this study.

7 Discontinuation of Study Intervention and Participant Discontinuation

7.1 Permanent Discontinuation of Study Intervention

It may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the study to be evaluated for the Day 14 and Day 28 safety follow-ups. See the SoA (Table 1-1) for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

Study intervention will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;

- Progresses to a level > 4 of the modified WHO ordinal scale.

Additionally, study intervention should be permanently discontinued in the following circumstances:

1. Discontinuation of study intervention for abnormal liver tests should be considered if the Investigator believes that it is in best interest of the participant. The development of alanine aminotransferase (ALT) ≥3 × ULN and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR is measured, which may indicate severe liver injury (according to Hy's Law), must be reported as an SAE and the study intervention discontinued.

2. If, as part of normal clinical follow-up, a clinically significant change in ECG is identified, the Investigators should exercise their clinical judgement to decide whether continuing study intervention administration is in the best interest of safety of the participant.

3. AE/SAE if the Investigator believes that it is in best interest of the participant, including the following:

- Development of significant bronchospasm * or worsening of cough that is not tolerated by the participant and leads to immediate discontinuation of the nebulization.

- Progressive COVID-19 requiring initiation of non-invasive or invasive ventilation or high flow oxygen.

- Development of mHb level above 3%.

- Inability to safely continue receiving study intervention due to compromised respiratory status while off supplemental oxygen and receiving nebulized therapy (manifest by drop in SpO_2, increased respiratory rate or clinical other findings).

Refer to the SoA (Table 1-1) for data to be collected at the time of study intervention discontinuation and follow-up and for any further evaluations that need to be completed.

** Inhaled citric acid and inhaled mannitol provoke bronchospasm in patients with bronchial hyperresponsiveness in a dose dependent pattern. Although the concentrations of mannitol and citric acid are below the provocative dose and concentration inducing bronchospasm, there is a theoretical risk that a participant may develop acute bronchospasm during the use of RESP301. Participants should therefore be monitored during RESP301 administration. In case of clinical*

intolerance or saturation decrease, nebulization should be immediately discontinued (see Section 7.2). Further clarification of the differences in signs and symptoms for acute bronchospasm and COVID-19 progression are provided in Table 7-1.

Table 7-1 Differences in Signs and Symptoms for Acute Bronchospasm and COVID-19 Progression

	Acute bronchospasm related to inhaled bronchial irritant	Progression of COVID-19 lung disease to acute respiratory failure
Mechanism	Acute contraction of bronchial muscles resulting in bronchial obstruction resulting in increased ventilatory load	Cytokine storm resulting in alveolar damage resulting in gas transfer impairment
Onset	Within minutes (<15 minutes) following inhalation (no late phase since it is not an allergic IgE mediated reaction)	Within hours or days and not related to nebulization
Clinical auscultatory signs	Wheezing (almost all cases)	Bilateral rales (inconstant)
Patient history	Asthma or respiratory allergy	Age > 65 years, increased BMI, high blood pressure, cardiac diseases, etc.
Reversibility with inhaled beta mimetics	Within minutes	No

Abbreviations: BMI=body mass index; IgE=immunoglobulin E.

7.2 Temporary Discontinuation of Study Intervention

Brief temporary discontinuation of study intervention is permitted during the study, providing inhalation of study intervention is completed within 20 minutes.

Study intervention should be temporarily discontinued in the following circumstance:

1. Clinical intolerance to nebulization.

   An individual administration of study treatment will be stopped, and supplemental oxygen resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Sections 4.2 and 8.1.2).

The participant may continue with subsequent doses if the Investigator judges that the benefit / risk ratio remains positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored.

Refer to Section 6.1 for information on staggered dosing in the first 10 participants.

7.3 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. The participant will be definitively discontinued from both the study intervention and from the study at that time.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

At the time of discontinuing from the study, if possible, an early discontinuation assessment should be conducted, as shown in the SoA (Table 1-1).

7.4 Loss of Participants to Follow-Up

A participant will be considered lost to follow-up if he or she is unable to be contacted by the study site. The following actions must be taken if a participant cannot be contacted at Day 14 or Day 28:

- The site must attempt to contact the participant and counsel the participant on the importance of maintaining the assigned schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- In cases in which the participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record/CRF.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 Study Assessments and Procedures

Study procedures and their timing are summarized in the SoA (Table 1-1). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Table 1-1).

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 20 mL.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Informed Consent

Informed consent must be documented according to Appendix 1, Section 10.1.3.

Eligibility Criteria

All inclusion and exclusion criteria will be reviewed by the Investigator or designee to ensure that the participant qualifies for the study.

Participant Identification Card

All participants will be given a participant identification card identifying them as participants in a research study. The card will contain study site contact information (including direct telephone numbers) to be used in the event of an emergency. The Investigator or qualified designee will provide the participant with a participant identification card immediately after the participant provides written informed consent. At the time of intervention allocation/randomization, site personnel will add the intervention/randomization number to the participant identification card.

Medical History

A medical history will be obtained by the Investigator or qualified designee. The medical history will collect all active conditions and any condition diagnosed that the Investigator considers to be

clinically relevant. Details regarding the disease for which the participant has enrolled in this study will be recorded separately and not listed as medical history.

Prior and Concomitant Medications Review

The Investigator or qualified designee will review prior medication use and record prior medications taken by the participant within 3 months prior to screening. This should include a history of hypertension medication, in particular angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

The Investigator or qualified designee will record medication, if any, taken by the participant during the study through the last assessment. Concomitant medications will be recorded on an ongoing basis during the study (or longer if related to an SAE).

8.1 Efficacy Assessments

8.1.1 Modified WHO Ordinal Scale

A modified WHO ordinal scale will be used for consistency with the recent study of lopinavir-ritonavir in adults hospitalized with severe COVID-19 (Cao et al, 2020), to record the participant's status at the time of assessment. The modified WHO ordinal scale includes the following levels:

1. Not hospitalized, no limitations on activities;
2. Not hospitalized, limitation on activities;
3. Hospitalized, not requiring supplemental oxygen;
4. Hospitalized, requiring supplemental oxygen;
5. Hospitalized, on non-invasive ventilation or high-flow oxygen devices;
6. Hospitalized, on invasive mechanical ventilation or extra corporeal membrane oxygenation (ECMO);
7. Death.

8.1.2 Pulse Oximetry

Pulse oximetry measurements will be performed to evaluate SpO_2 as outlined in the SoA (Table 1-1) and in accordance with the site standard operating procedures, on a medical-grade medical device. Measurements will be taken with a probe on fingertip or earlobe and recorded as

percent oxygenated hemoglobin. Supplemental oxygen used at the time of assessment and method of oxygen delivery will be collected and documented along with the SpO_2.

Prior to administration of study intervention, supplemental oxygen will be held for at least a minute and SpO_2 checked in order to ensure participant may safely receive the study intervention. For safety reasons, since due to the current pandemic it cannot be guaranteed that study intervention will be consistently administered under direct medical supervision, the study Investigators should provide study personnel who are able to monitor the participant and their SpO_2 and ensure that should it decrease to a threshold, this would trigger immediate discontinuation. By default, unless overruled by the study Investigator, this SpO_2 threshold is set to 89%. This threshold should be entered into the saturation monitoring device to trigger an alarm during nebulization.

FiO_2 will be measured per site standard practice and recorded in the eCRF. The device used to administer oxygen should also be recorded.

8.1.3 National Early Warning Score (NEWS) 2

The NEWS 2 will be measured prior to study intervention in the morning according to the SoA in Table 1-1.

The NEWS is based on a simple aggregate scoring system in which a score is allocated to physiological measurements, already recorded in routine practice, when patients present to, or are being monitored in hospital (RCP, 2012). Six simple physiological parameters form the basis of the scoring system:

1. Respiration rate
2. Oxygen saturation
3. Systolic blood pressure
4. Pulse rate
5. Level of consciousness or new confusion*
6. Temperature

**The patient has new-onset confusion, disorientation and/or agitation, where previously their mental state was normal – this may be subtle. The patient may respond to questions coherently, but there is some confusion, disorientation and/or agitation. This would score 3 or 4 on the Glasgow Coma Scale (rather than the normal 5 for verbal response), and scores 3 on the NEWS system.*

A score is allocated to each parameter as they are measured, with the magnitude of the score reflecting how extremely the parameter varies from the norm (zero for 'normal'; maximum score 3). The score is then aggregated. The score is increased by 2 points for people requiring supplemental oxygen to maintain their recommended oxygen saturation. This is a pragmatic approach, with a key emphasis on system-wide standardization and the use of physiological parameters that are already routinely measured in National Health Service (NHS) hospitals and in prehospital care, recorded on a standardized clinical chart – the NEWS 2 chart (Refer to Appendix 2: The NEWS 2 Scoring System).

Reproduced from: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017 (RCP, 2017).

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1-1).

8.2.1 Physical Examinations

A complete physical examination will include, at a minimum, assessments of the head/ear/eyes/nose/throat, cardiovascular, respiratory, gastrointestinal, lymphatic, skin and neurological systems. Height (screening only) and weight will also be measured and recorded.

A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

8.2.2 Vital Signs

Vital signs to be collected as outlined in the SoA (Table 1-1) and include body temperature, heart rate, blood pressure, and respiratory rate.

Body temperature will be assessed per the local practice (temporal or otic are preferred sites), and site will be recorded. Pulse rate, respiratory rate, and blood pressure will also be assessed per site SOC. Where possible the same methods should be used throughout the study for an individual participant.

Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones). During study intervention administration, an alarm threshold should be set for heart rate at 120 beats/min (or as indicated by the Investigator), as outlined in the SoA (Table 1-1).

Vital signs (to be taken before blood collection for laboratory tests) will be measured after the participant has been sitting for 5 minutes.

8.2.3 Electrocardiograms

Single 12-lead ECG will be obtained as outlined in the SoA (Table 1-1) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals for local reading.

8.2.4 Clinical Safety Laboratory Assessments

Refer to Appendix 3 for the list of clinical laboratory tests to be performed and to the SoA (Table 1-1) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically relevant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically abnormal during participation in the study or within 4 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, where possible, and the Sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the Laboratory Manual and the SoA (Table 1-1).
- If laboratory values from laboratory assessments not specified in the protocol and performed at the institution’s local laboratory result in the need for a change in participant management or are considered clinically relevant by the Investigator (e.g., are considered to be an SAE or an AE), then the results must be recorded in the CRF.

8.3 Adverse Events and Serious Adverse Events

The definitions of AEs and SAEs can be found in Appendix 4.

Adverse events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or the study, or that caused the participant to discontinue the study intervention (see Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from signing of informed consent until the last follow-up visit at the time points specified in the SoA (Table 1-1).

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours of the Investigator becoming aware of the SAE, as indicated in Appendix 4. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obliged to actively seek AEs or SAEs after the conclusion of study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.3.2 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is given in Appendix 4.

8.3.4 Regulatory Reporting Requirements for SAE

Prompt notification (within 24 hours, see Appendix 4) by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and Investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAE) from the Sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

All women, including women of childbearing potential are allowed in the study. Urine pregnancy test will be performed on female participants of childbearing potential and pregnant females at screening. Females who are not of child bearing potential do not need to undergo a screening pregnancy test.

Pregnant females will be followed to determine the outcome of the pregnancy:

- The Investigator will collect any follow-up information on the participant and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of the pregnancy will be reported, regardless of fetal state (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered to be SAEs. Any post-study pregnancy-related SAE considered reasonably related to study intervention by the Investigator will be reported to the Sponsor as described in Section 8.3.4. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related events (DREs) are common in participants with COVID-19 and can be serious/life-threatening:

- Fever
- Cough*
- Dyspnea*
- Asthenia
- Loss of sense of taste and smell

** A cough or dyspnea episode related to study intervention administration does not meet the definition of a DRE and should be reported as an AE.*

Because these events are typically associated with the disease under study, they will not be reported as AEs.

NOTE: However, if either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the Investigator's opinion, of greater intensity, frequency, or duration than expected for the individual participant.

  OR

- The Investigator considers that there is a reasonable possibility that the event was related to study intervention.

8.3.7 Adverse Events of Special Interest (Not Applicable)

No AEs of special interest are defined for this study.

8.3.8 Medical Device Deficiencies (Not Applicable)

The medical device to be used in this study is a vibrating mesh nebulizer that is FDA-approved and CE marked and is to be used per the manufacturer's recommendations. Therefore, data on device deficiencies will not be collected in this study.

8.4 Tokenization (Optional)

Tokenization is the process of converting a piece of data into a random string of characters known as a token. Tokenization protects sensitive data by substituting non-sensitive data. The

token serves merely as a reference to the original data, but cannot be utilized to determine those values. The advantage of tokens is that there is no mathematical relationship to the real data that they represent. The real data values cannot be obtained through reversal, and hence, a breach renders the information invaluable. Tokens are being increasingly used to secure varying types of sensitive information. In particular, personal identifiable information such as healthcare information, email addresses and account numbers are such examples. From a security perspective, tokenization significantly reduces risk based on the fact that sensitive data cannot be breached if it is not there in the first place.

Tokenization applies only to US participants who agree and sign the optional ICF. The piece of personal data needed to generate the token will be collected to create a unique, de-identified token. This token would be instrumental to enrich and aggregate other study participants' data coming from different sources for the purpose of future research. The benefit of linking data in general, is that the data set that is created can be used to answer a variety of healthcare- and therapy-related questions that could not otherwise be answered through conventional means.

8.5 Treatment of Overdose (Not Applicable)

There is no risk of overdose.

8.6 Pharmacokinetics (Not Applicable)

Pharmacokinetic parameters are not evaluated in this study.

8.7 Pharmacodynamics (Not Applicable)

Pharmacodynamic parameters are not evaluated in this study.

8.8 Genetics (Not Applicable)

Pharmacogenomics are not evaluated in this study.

8.9 Biomarkers (Not Applicable)

Biomarkers are not evaluated in this study.

8.10 Medical Resource Utilization and Health Economics (Not Applicable)

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 Statistical Considerations

9.1 Statistical Hypothesis

RESP301 reduces the rate of progression to level 5 and above in the modified WHO ordinal scale in COVID-19 (see Section 8.1.1).

This represents a composite endpoint of (i) death (level 7), (ii) hospitalized, on invasive mechanical ventilation or ECMO (level 6), or (iii) hospitalized, on non-invasive ventilation or high-flow oxygen devices (level 5).

9.2 Sample Size Determination

A total of 300 hospitalized participants with confirmed COVID-19 will be randomized 2:1 to receive RESP301+SOC (200 participants) or SOC alone (100 participants).

The comparison between the treatment arms for the primary endpoint will have approximately 80% power and alpha level 0.025 (one-sided) to demonstrate significant reductions (15% versus 30%) of the primary endpoint between RESP001+SOC and the Control arm (SOC), a 50% relative reduction for the proportion of participants who progress to level >4 (see definition of the primary endpoint).

Two interim analyses are planned (see Section 9.5 for details):

1. The first IDMC interim analysis will take place after the first 60 participants have completed Day 10 of the study to evaluate whether the study can be stopped for futility based on change from baseline in room air SpO_2. For a final decision to stop the study for futility the results on other endpoints will be considered as well.
2. The second interim analysis will take place after 150 participants have completed Day 14 post-randomization based on event rate for the primary endpoint. The purpose of the second interim analysis will be futility as well as a potential sample size re-estimation in case the actual results differ from the original assumptions.

In addition to the review of efficacy data, safety will be assessed at each of the interim analysis by the IDMC. Further details are provided in Appendix 5.

9.3 Populations for Analyses

For purposes of analysis, the following analysis sets are defined:

Table 9-1 Populations for Analysis

Population (Analysis Set)	Description
Intent-To-Treat (ITT) Population	The ITT Population will include all randomized participants. The ITT participants will be analyzed according to randomized treatment, irrespective of the actual treatment received. Participants who withdraw from treatment early will be followed for the assessment of the Day 14 primary endpoint. All efficacy analyses will be performed using the ITT Population.
Per Protocol (PP) Population	The PP Population will include all participants in the ITT Population with no major protocol deviations that may significantly impact data integrity or patient safety. The PP Population will be used for supportive analyses of the efficacy measurements.
Safety Population (SP)	The SP will include all randomized participants who inhale any amount of study intervention or are randomized to the control arm. The SP will be analyzed according to the actual treatment received. This set will be used for the safety analyses.

The ITT Population will be the primary analysis set for all efficacy analyses and the PP Population will be used to demonstrate robustness of results for the primary efficacy endpoint.

9.4 Statistical Analyses

Below is a description of planned statistical analyses. Further details are presented in the Statistical Analysis Plan (SAP).

9.4.1 General Considerations

All statistical analyses will be conducted using SAS, Version 9.4 or later. Baseline characteristics will be summarized by treatment arm. For continuous measures the mean and standard deviation (SD) will be summarized. Categorical variables will be described by the proportion in each category. In addition, 95% confidence intervals (CIs) will be computed as indicated.

All the categorical variables including the primary endpoint will be summarized by treatment with the numbers and percentages of the participants. Treatment difference will be tested using a Cochran–Mantel–Haenszel test stratified by 1) country and 2) participant's clinical status at Baseline. For the categorical endpoints, relative risk and its 95% CI will be presented.

All of the continuous variables, including the changes from Baseline, will be summarized by treatment with the means, SD, medians and the ranges. The mixed model with repeated

measurements /analysis of covariance model with treatment, country, participant's clinical status at Baseline and visit as the model term, and Baseline value as the covariate will be used to test for the significance of the treatment difference. Least square means, standard errors, 95% CIs and p-values will be presented.

Time to event endpoints will be analyzed using the Cox Proportional Hazard model with treatment, participant's clinical status at Baseline and country as the model term. The hazard ratio of RESP301+SOC versus SOC will be presented along with 95% CI and p-value from the model. The Kaplan-Meier curves of the time to events will be presented by treatment for each applicable endpoint.

Handling of missing data

If participants are discharged from the hospital prior to Day 14 due to improvement of the clinical status and their status on Day 14 cannot be obtained, their status on Day 14 for the primary endpoint will be imputed with the status on the day of discharge. Depending on the reasons for missing data on the primary endpoint up to Day 14, additional sensitivity analyses will be performed. Further details on handling on missing data will be provided in the SAP.

9.4.2 Primary Endpoint

The primary endpoint is the proportion of participants who progress to level >4 of the modified WHO ordinal scale due to COVID-19 by Day 14.

9.4.3 Secondary Endpoint(s)

The key secondary endpoints are:

1. Change from baseline on the modified WHO ordinal scale at each visit up to Day 28
2. Change in room air SpO_2 from baseline over time
3. Change in NEWS 2 symptom score from baseline over time
4. Time to improvement to a lower level (<4) of the modified WHO ordinal scale
5. Time to progression to a higher level (>4) of the modified WHO ordinal scale

Additional secondary endpoints are:

1. Time to hospital discharge
2. Incidence of mortality by Day 28

9.4.4 Tertiary/Exploratory Endpoint(s)

CCI

- CCI

9.4.5 Other Safety Analyses

All safety analyses will be performed on the Safety Population.

1. Safety and tolerability assessed by clinical safety laboratory measurements, physical examinations, vital signs, concomitant medications; cumulative incidence of AEs, SAEs and severe AEs
2. Incidence of participants unable to tolerate nebulization due to:
    - Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
    - Other clinical signs of intolerance according to Investigator's opinion
3. Incidence of clinical bronchial hyper-responsiveness related to nebulization

9.4.5.1 Adverse Events

Adverse Events will be coded using the MedDRA coding dictionary.

The number and percentage of participants with any AE, any related AE, any SAE, any related SAE, any severe AE, and related severe AE as well as the total number of events for each category will be summarized. The number of deaths due to an AE, hospitalization due to an AE, and study discontinuation due to an AE will be summarized.

The number and percentage of participants with an AE, as well as the total number of AEs, will be summarized by SOC and preferred term. This tabulation will be repeated for related AEs, SAEs, related SAEs, severe AEs, and related severe AEs.

All AEs will be provided in patient listings. Patient listings of AEs causing discontinuation of study medication, AEs leading to death, SAEs, related AEs, and severe AEs will be produced.

9.4.5.2 Clinical Laboratory Evaluation

Baseline is defined as the last non-missing value obtained at the screening visit and prior to the first exposure to study drug. Actual values and changes from Baseline clinical laboratory tests

will be summarized by study day. If more than one laboratory result is reported per study visit per parameter, the last non-missing result will be selected for change from Baseline analysis.

Laboratory test results will be classified according to the reference ranges and clinical significance as determined by the Investigator. The number of participants with a non-missing result, the number and percentage of participants with a clinically significant result less than the lower limit of normal, non-clinically significant result more than the ULN, and clinically significant result more than the ULN will be summarized by study visit. If more than one laboratory result is reported per study day per parameter, the result yielding the most severe classification will be selected for analysis.

Categorical laboratory test results (urinalysis excluding pH) will be summarized by study visit. If more than one laboratory result is reported per study day per parameters, the result yielding the most severe classification will be selected for analysis.

Participants who had urine pregnancy test at screening and the results will be listed.

Participants with clinically significant abnormal laboratory test results will be listed. This listing will include all results of the laboratory parameter that was abnormal and determined to be clinically significant by the Investigator for a participant across study visit.

9.4.5.3 Vital Signs

Baseline is defined as the last non-missing value obtained in screening and prior to the first exposure to study drug. Actual values and changes from Baseline in vital signs will be summarized by study day and study time point. All vital sign data will be presented in patient listings.

Vital sign values will be classified according to the clinical significance as determined by the Investigator. The number of participants with a non-missing result, the number and percentage of participants with a non-clinically significant result, and clinically significant result will be summarized by study visit and study time point. If more than one vital sign result is reported per study day and study time point per parameter, the result yielding the most severe classification will be selected for analysis.

Participants with clinically significant vital sign values will be listed. This listing will include all results of the vital sign parameter that was determined by the Investigator to be clinically significant for a participant across study time points.

9.4.5.4 Physical Examination

Abnormal physical examination findings will be listed.

9.4.6 Other Analyses

Other analyses may be added to the SAP as applicable.

9.5 Interim Analyses

Two interim analyses will be performed.

1. The first analysis will be conducted when about 60 participants (40 participants in the RESP301+SOC arm and 20 participants in the SOC arm) have completed the Day 10 assessment. The purpose of this analysis is to evaluate efficacy of RESP301 with the application of a futility criterion based on the results on SpO_2 change from baseline on Day 10. The following futility criterion will be used for this first interim analysis:

   If the difference in the percentage of participants with at least a 2% improvement in SpO_2 between both treatment arms is less than 5%, benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

   As no other modification of the study at the time of the first interim analysis is considered, no adjustment of the alpha level is required.

2. The second interim analysis will be conducted after 150 participants (100 participants in the RESP301+SOC arm and 50 participants in the SOC arm) have completed the Day 14 assessment. The purpose of this analysis is the assessment of futility or a sample size re-estimation in case the actual results differ from the original assumptions for the power calculations of the study, related to the percentage of participants meeting the primary endpoint in the control arm and/or the relative treatment benefit achieved in the RESP301+SOC arm compared to the SOC arm.

   To account for the multiple testing due to the second interim analysis an adjustment for the type I error alpha will be applied using the Haybittle-Peto approach which would spend one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis. The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration

will be given to stop the study for futility. At the same time sample size re-estimation will be performed to achieve 80% power at the end of the study. The methodology for the adjustment and the procedure to maintain the type I error level will be described in greater detail in the SAP.

Detailed information, including the boundaries futility and characteristics for the sample size re-estimation at the time of the interim analyses will be provided in the SAP and Data Monitoring Committee (DMC) Charter. Further details are also provided in Appendix 5.

9.6 Data Monitoring Committee (DMC)

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet to review after 10 and 20 participants have completed as well as at the time of the interim analyses (ie, after 60 and 150 participants have completed; see Section 9.5).

Due to the urgency of the pandemic, if participants become eligible for the study during the IDMC review of the first 10 participants then they may be recruited. However, their administration of RESP301 will be staggered in the same way as for the first 10 participants (see also Section 6.1). Once the IDMC review has been completed, and provided there are no safety concerns, further recruitment will continue as per protocol. The Sponsor and CRO confirm that the report from the IDMC will be sent in a timely manner to all participating sites and investigators.

The detailed procedures and criteria of the interim analyses will be described in the DMC Charter.

10 Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
    - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
    - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The Investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC.
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures.
    - Overall conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH GCP guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Financial Disclosure

Information on financial disclosure can be found in the Investigator Site File.

10.1.3 Informed Consent Process

The Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IEC/IRB and signed by all participants subsequently enrolled in the study as well as those currently enrolled in the study.

For US participants who agree to tokenization, a separate optional ICF will be provided.

10.1.4 Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Data Protection for Tokenization information is addressed in the separate optional ICF (for US participants only).

10.1.5 Committees Structure

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study (see Sections 9.5 and 9.6).

10.1.6 Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategies (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations [CROs]).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for at least 2 years after the last approval of a marketing application or 15 years from completion of the study, whichever is longer according to the relevant local laws and/or regulations. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

- All data generated by the site personnel will be captured electronically at each study center using eCRFs. Data from external sources (such as laboratory data) will be imported into the database. Once the eCRF clinical data have been submitted to the central server at the independent data center, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.
- If additional corrections are needed, the responsible monitor or data manager will raise a query in the electronic data capture (EDC) application. The appropriate staff at the study site will answer queries sent to the Investigator. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will freeze the eCRF page.
- The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the EDC system/eCRF.

10.1.7 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

10.1.8 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the first participant signing the informed consent form and will be the study start date.

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the Investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up

10.1.9 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor at least one month before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.10 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first participant is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/Competent Authority approval prior to implementation (if appropriate). In the US: Following approval, the protocol

amendment(s) will be submitted to the Investigational New Drug application under which the study is being conducted.

Administrative changes (not affecting the participant benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.1.11 Liability and Insurance

10.1.11.1 Access to Source Data

Access to source data is described in the Clinical Site contract.

10.2 Appendix 2: The NEWS 2 Scoring System

Chart 1: The NEWS 2 scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

Abbreviations: CVPU: C=new onset confusion, disorientation or agitation, V=responds to voice, P=responds to pain, U=unresponsive; SpO_2=oxygen saturation.

Source: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2

10.3 Appendix 3: Clinical Laboratory Tests

The tests detailed in Table 1-1 will be performed by the local laboratory.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol. Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 10-1 Protocol-Required Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	CBC without differential: White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet, mean platelet volume Methemoglobin, nitrite			
Clinical Chemistry[1]	Blood urea nitrogen Creatinine Glucose non-fasting	Potassium Sodium Chloride Bicarbonate/CO_2	Aspartate Aminotransferase / Serum Glutamic-Oxaloacetic Transaminase Alanine Aminotransferase/ Serum Glutamic-Pyruvic Transaminase Alkaline phosphatase / Lactate dehydrogenase	Total and direct bilirubin Coagulation (PT/INR, aPTT)
Other Screening Tests	Highly sensitive urine human chorionic gonadotropin pregnancy test (for women of childbearing potential and pregnant women) [2]			

Laboratory Assessments	Parameters
	The results of each test must be entered into the eCRF.

NOTES:

1 Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1. All events of ALT ≥3 × ULN and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR is measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE and the study intervention discontinued.

2 Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

Investigators must document their review of each laboratory safety report.

10.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1 Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (e.g., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.4.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization (Applies only during the safety follow up period for participants who may have been discharged during the treatment period, ie, between Day 1 and Day 10)

- In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or intervention that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are considered AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective intervention of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive intervention in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.4.3 Recording and Follow-up of AE and SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event. • The Investigator will then record all relevant AE/SAE information in the CRF. • It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to the clinical research organization (CRO) in lieu of completion of the AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by the CRO. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to Parexel Safety Services for review. • The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories: • Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities. • Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe. An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality
• The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. • A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The Investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated. • The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment. • For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report in the electronic CRF/EDC. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to electronic CRF/EDC.** • The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. • The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE
• The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the DMC to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • New or updated information will be recorded in the originally completed CRF.

- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.4.4 Reporting of SAE

SAE Reporting to Parexel Safety Services via Electronic Data Collection Tool

The Investigator must report any SAEs to the Parexel Safety Services within 24 hours of becoming aware of the event.

All SAEs will be recorded from signing of informed consent until the end of the study. Serious adverse events occurring after the end of the study and coming to the attention of the Investigator must be reported only if they are considered (in the opinion of the Investigator) causally related to study intervention.

- The primary mechanism for reporting an SAE to Parexel Safety Services will be the electronic data collection tool.
- The site will additionally use the paper SAE data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- The Investigator and the Sponsor (or Sponsor's designated agent) will review each SAE report and the Sponsor/Parexel will evaluate the seriousness and the causal relationship of the event to study intervention. In addition, the Sponsor (or Sponsor's designated agent) will evaluate the expectedness according to the reference document (Investigator Brochure or Summary of Product Characteristics). Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by email.
- Contacts for SAE reporting can be found in the Investigator Manual.

Suspected Unexpected Serious Adverse Reactions (SUSARs)
Any AE that is a SUSAR has additional reporting requirements, as described below. • If the SUSAR is fatal or life-threatening, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within seven calendar days after the Sponsor learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information should be reported within an additional 8 days (15 days total). • If the SUSAR is not fatal or life-threatening but is otherwise serious, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within 15 calendar days after the Sponsor learns of the event. The Sponsor will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of participants. Follow-up information may be submitted if necessary. The Sponsor will also provide annual safety updates to the regulatory authorities and IECs responsible for the study. These updates will include information on SUSARs and other relevant safety findings.

All SAEs that occur during the study, and all SAEs occurring up to 18 days after receiving the last dose of study intervention, whether considered to be associated with the study intervention or not, must be reported within 24 hours via electronic data collection tool and paper data collection tool to Parexel Safety Services.

The minimum information required for an initial report is:

Name of person sending the report (e.g., name, address of Investigator);

- Participant identification (screening/randomization number, initials, NOT participant name);
- Protocol number;
- Description of SAE;
- Causality assessment, if possible.

However, as far as possible all points on the SAE form should be covered in the initial report, or the completed SAE form itself must be emailed or faxed to the Parexel Safety Services. In addition, the event must be documented in the electronic CRF/EDC system.

After receipt of the initial report, Parexel Safety Services will review the information and, if necessary, contact the Investigator, to obtain further information for assessment of the event. Parexel will be responsible for all information processing and reporting according to local legal requirements. Where necessary, Investigators will inform regulatory authorities in their own countries.

10.5 Appendix 5: Statistical Design Considerations

10.5.1 Some General Design Considerations

1. Binary primary endpoint: progression to critical stage/death up to Day 14
    - to be analyzed by a Cochran-Mantel-Haenszel (CMH) stratified for the factors used in the stratified randomization
    - subjects who withdraw from study treatment will be followed up until their primary endpoint outcome is known (i.e., up to Day 14 or progression to critical stage/death, whatever is first)
    - it is, therefore, not clear what current protocol synopsis text could mean: *If subjects are discharged from the hospital prior to day 14 due to improvement of the clinical status and their status on day 14 cannot be obtained, their status on day 14 for the primary endpoint will be imputed with the status on the day of discharge. and what the implications on statistical study characteristics could be. Further discussion is needed.*
2. Timing of the two planned interim analyses with stopping options is stated as

There are 2 interim analyses planned, the first interim analysis after the first 60 participants have completed day 10 of the observation period … and a second interim analysis after 150 participants have completed day 14 of the observation period.

With the current text for the timing of the first interim analysis, it is not clear how many subjects will have been randomized at least 14 days prior to the interim data cut-off date and can therefore provide data for the binary primary endpoint ("progression to critical stage/death up to Day 14").

For such a binary endpoint, Parexel does not recommend including any participant randomized less than 14 days prior to the interim data cut-off date (note this would be different to a study with a time-to-event endpoint) as

- for such participants not yet progressed, the outcome up to Day 14 is unknown and should not be imputed as "not progressed up to Day 14"
- for such participants progressed prior to Day 14, the outcome is known but their inclusion would bias the estimation of progression probability up to Day 14 in a upwards direction.

In order to set up the adaptive group-sequential study design, the following assumptions have been used:

- first interim analysis: a total of ≈ 39 participants randomized at least 14 days prior to data cut-off date
- second interim analysis: a total of ≈ 50% of the initially planned number of participants randomized at least 14 days prior to data cut-off date.

3. Non-binding DMC guidance for stopping the study for futility at the first interim analysis is stated as:

If the difference in the percentage of participants with at least a 2% improvement in SpO2 between both treatment arms is less than 5%, a benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

As the main futility stopping criterion is based on improvement in SpO_2 (and not on the primary efficacy endpoint), this first interim analysis will be "ignored" in these statistical considerations for an adaptive group-sequential design. We may revisit the first interim analysis at a later point in time when more information is available.

4. Non-binding DMC guidance for stopping the study for futility at the second interim analysis stated as:

The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration will be given to stop the study for futility.

5. Current DMC guidance for stopping the study for superior efficacy at the second interim analysis is stated as:

To account for the multiple testing due to the second interim analysis an adjustment for the type-I-error probability will be applied using the Haybittle-Peto approach with one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis.

6. For initial sample size calculation, the following additional assumptions / features from the protocol have been used

- progression probability up to Day 14 for Standard of Care (SOC): 30%

- progression probability up to Day 14 for RESP301+SOC: 15%
- desired power assuming the alternative hypothesis stated above: 80%
- 2:1 randomization

7. Method for adaptation of the sample size based on the second interim analysis data is stated as

A sample size re-estimation will be performed to achieve 80% conditional power at the end of the study. The methodology for the adjustment and the procedure to maintain the type-I-error level will be described in greater detail in an appendix to the protocol. The maximum increase of the sample size will be limited to a total number of 600 randomized subjects.

Parexel has put a suggestions with some fictitious interim data for illustration in Section 10.5.3; that section also illustrates by simulations the Cui/Hung/Wang (CWH) approach using a weighted (weights fixed at the design-stage) test for the final analysis versus the Chen/DeMets/Lan (CDL) approach without such weights at the final analysis.

10.5.2 General Design Characteristics

Parexel proposes to base non-binding futility stopping criteria on conditional power criteria. Conditional power (CP) is the conditional probability for achieving statistically significant superiority of RESP301+SOC over SOC at the final analysis, given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study (an alternative would be predictive power (PP), a Bayesian version of CP where prior distributions and observed interim results are combined to obtain the probability for achieving statistically significant superiority of gimsilumab over placebo at the final analysis).

Randomization 2:1	**Group-Sequential Design**
1st Interim Analysis (IA) 2nd IA	N ≈ 39 for D14 ≈ 50% for D14
Futility criterion 1st IA Futility criterion 2nd IA Superior efficacy criterion 2nd IA	Based on SpO_2 CP < 4% p < 0.0005
Total sample size required for 80% power	**300**
Futility stopping probability at 2nd IA under "No effect" Futility stopping probability at 2nd IA under "Planned effect 30% vs 15%"	1st IA: **NA** 2nd IA: **70%** 1st IA: **NA** 2nd IA: **6%**
Superiority stopping probability at 2nd IA under "Planned effect 30% vs 15%" Superiority stopping probability at 2nd IA under "30% vs 10%"	**10%** **28%**

10.5.3 Adaptive Sample Size Re-Estimation

Another objective of the 2nd interim analysis is to re-estimate the sample size based on the unblinded interim results.

Proposed procedure at 2nd interim analysis (planned to include approximately 150 subjects)

- unblinded analysis of the primary efficacy endpoint ("progression to level >4 of modified WHO ordinal COVID-19 scale by Day 14" as a binary endpoint)
- calculation of conditional power (CP) given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study
    - if CP < 4%: recommend early stop for futility
    - if one-sided p-value < 0.0005: recommend early stop for superior efficacy
    - if 50% < CP < 80% and one-sided p-value ≥ 0.0005:
        - continue the study with an increased total sample size N*, so that CP with a total of N* subjects is increased to 80%, same value as the (unconditional) desired power in the study design
        - N*, however, is limited to 600 (which is twice the original sample size 300)
    - if 4% ≤ CP ≤ 50%: continue the study without a change in sample size (otherwise, the sample size would need to be increased too much or the maximum sample size of 600 would not be sufficient to come close to a CP of 80%.

The table on the next page illustrated the proposed procedure for a number of possible results observed at the 2nd interim analysis.

Table with examples for the adaptive sample size procedure described above using the Cui/Hung/Wang (CHW) approach using a weighted (fixed weights determined by the stage-wise sample sizes planned at the design-stage) test for the final analysis:

2nd IA results with n=150 subjects (50 for SOC, 100 for RESP301+SOC		**CP under observed trend for planned 300**	**Total sample size required for CP 80% under observed trend**	**CP under observed trend if total sample size capped by 600**
15 (30%)	5 (5%)	$p < 0.0005$ → early stop for superior efficacy		
15 (30%)	10 (10%)	99%	Not applicable	Not applicable
15 (30%)	15 (15%)	90%	Not applicable	Not applicable
15 (30%)	18 (18%)	66%	393	Not applicable
15 (30%)	19 (19%)	55%	483	Not applicable
15 (30%)	21 (21%)	34%	[798]	[67%]
15 (30%)	26 (26%)	3.99%	Early stop for futility	
12 (24%)	10 (10%)	92%	Not applicable	Not applicable
12 (24%)	12 (12%)	77%	321	Not applicable
12 (24%)	14 (14%)	54%	498	Not applicable
12 (24%)	15 (15%)	42%	[646]	[77%]
12 (24%)	18 (18%)	14%	[1800]	[31%]
12 (24%)	20 (20%)	2.6%	Early stop for futility	
10 (20%)	8 (8%)	85%	Not applicable	Not applicable
10 (20%)	10 (10%)	64%	412	Not applicable
10 (20%)	11 (11%)	50.4%	534	Not applicable

10 (20%)	13 (13%)	26%	[1014]	[55%]
10 (20%)	15 (15%)	10%	[2460]	[23%]
10 (20%)	18 (18%)	1.4%	Early stop for futility	

Quantities in [ ] indicate theoretical values as sample size would not be increased per the currently proposed adaptive design.

10.5.4 Overall Adaptive Design Performance

Finally, the Cui/Hung/Wang (CHW) approach is compared to the Chen/DeMets/Lan (CDL) approach without pre-defined fixed weights for the final analysis (which is valid as sample size may only be increased if CP (obtained under observed trend) at the second interim analysis exceeds 50%).

All the results below are for the group-sequential design with adaptive sample size re-estimation procedure as described in previous sections and based on 10.000 simulated trials each.

Scenario 1: true progression probabilities are 30% (SOC) and 15% (RESP301+SOC)

Scenario 2: true progression probabilities are 24% (SOC) and 14% (RESP301+SOC)

Scenario 3: true progression probabilities are 30% (SOC) and 10% (RESP301+SOC)

Scenario 4: true progression probabilities are 24% (SOC) and 20% (RESP301+SOC)

10.6 Appendix 6: Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CBC	complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CP	Conditional power
CRF	case report form(s) (paper or electronic as appropriate for this study)
CRO	contract research organization
CV	cardiovascular
DMC	Data Monitoring Committee
DRE	disease-related events
ECG	electrocardiogram
ECMO	Extra corporeal membrane oxygenation
EDC	electronic data capture
EoS	End of study
FDA	Food and Drug Administration
FiO_2	fraction of inspired oxygen
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IVRS	interactive voice response system

IWRS	interactive web response system
LFT	Liver function test
mHb	Methemoglobin
NEWS	National Early Warning Score
NHS	National Health Service
NO	Nitric oxide
NO_2	Nitrogen dioxide
NOx	Oxide of nitrogen
RT-PCR	Reverse transcriptase polymerase chain reaction
PP	Per protocol
PT	Prothrombin time
QTcF	QT interval corrected using Fridericia's formula
SAE	serious adverse event
SAP	statistical analysis plan
SD	Standard deviation
SoA	schedule of activities
SOC	Standard of care
SP	Safety population
SpO_2	Oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
TID	Three times daily
ULN	Upper limit of normal
US	United States
WHO	World Health Organization

10.7 Appendix 7: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

10.7.1 Protocol Amendment 1.0, 05 Jun 2020

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the EU.

Overall Rationale for the Amendment:

The original protocol was updated to include important safety guidelines for study intervention administration for the first 10 participants (ie, up to the first independent data monitoring committee [IDMC] safety review). It was also updated to align with the IB Version 2.0, dated 27 May 2020. Other changes, with brief rationale, are summarized in the following table.

Section # and Name	Description of Change	Brief Rationale
Global change	Device name Philips InnoSpire Go was replaced with generic reference to a vibrating mesh nebulizer.	The decision was made to keep the device generic for flexibility.
Section 1.1 Synopsis and Section 4.1 Overall Design	Clarified that participants were (male or female) adults. Wording of study design also clarified to include study intervention and minor clarification to text for screening period.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 1.1 Synopsis, Section 4.1 Overall Design, Section 7.1, Discontinuation of Study Intervention	Bullets specifying reasons for permanent discontinuation updated to reflect content of Section 7.1.	Correction, for consistency across the protocol.

Section # and Name	Description of Change	Brief Rationale
Section 1.2 Schema	Dose of study intervention corrected to 6.0 mL. Minor clarifications added in footnotes.	Correction.
Section 1.2 Schema	Footnote 2 updated.	Text added to make it clear that participants will be allowed sufficient time to consider their participation in the study.
Section 1.1 Synopsis, Section 1.3 Schedule of Activities (SoA), Section 2.2.1 Risk Assessment, Section 4.1 Overall Design, and Section 6.1 Study Intervention Administered	The following statement was added: A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). RESP301 will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6). The time gap between consecutive doses in the 3 times per day (TID) schedule was updated to “at least 6 hours” from “at least 4 hours”.	Updated to align with the Investigator Brochure (IB) Version 2.0, dated 27 May 2020.
Section 1.3 SoA, Section 5.2 Inclusion Criteria, Section 8.3.5 Pregnancy, Appendix 3, Clinical Laboratory Tests	Addition of urine pregnancy test at screening. Requirement for all females of childbearing potential, including pregnant females, to consent to urine pregnancy testing at screening. Details of pregnancy follow-up procedures added.	All women, including women of childbearing potential and pregnant women, are allowed in the study. Therefore, it is necessary to determine pregnancy status at study entry so that pregnant females can be followed to determine the outcome of the pregnancy.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 SoA, Appendix 3, Clinical Laboratory Tests	Deletion of tuberculosis (TB) screening test. Nitrite added to the protocol-required laboratory assessments (End of Study [EoS] only).	Screening TB test is not mandatory for this study. Nitrite is to be measured for participants who are still hospitalized at EoS.
Section 1.3 SoA and Section 7.1 Permanent discontinuation of Study Intervention	Deletion of electrocardiogram (ECG) testing after screening. Clarified that significant changes in ECGs would be identified only as part of normal clinical follow-up.	Only routine ECG monitoring is needed for this study.
Section 2.1 Background, Section 11 References	Text deleted from end of paragraph 12. References no longer cited were removed from Section 11.	Removal of text that was specific to the Philips InnoSpire Go nebulizer.
Table 2-1, Risk Assessment and Section 11 References	The risk assessment table was updated to align with the above changes and IB. A new reference (Brannan et al, 2005) was added to Section 11 to support additional text around risk of bronchospasm.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 1.1 Synopsis, Section 1.3 SoA, Section 4.1, Overall Design	Visit window for Day 14 and Day 28 follow-up extended to (± 2 days).	To allow more flexibility.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 SoA	Footnote 2 corrected to include weight and exclude ECG. Clarified that laboratory testing at EoS follow up is for nitrite and methemoglobin only.	Correction and clarification.
Section 1.3 SoA	Footnote 6e corrected from 'Recording of SpO_2 * pre-nebulization on ambient air' to 'Recording of SpO_2 * on ambient air'.	'pre-nebulization' was removed as SpO_2 measurement on ambient air is required for both active and control group.
Section 1.3 SoA	Footnote 7 amended to clarify text that is applicable to participants in the active arm only.	Clarification.
Section 1.3 SoA, Section 8.3 Adverse Events and Serious Adverse Events, Section 8.3.8 Medical Device Deficiencies, and Appendix 5: Medical Device Adverse Events (AEs) Adverse Device Effects (ADEs), Serious Adverse Events (SAEs) and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-up, and Reporting	Changes related to the removal of Appendix 5. Table 8-1 deleted.	Appendix and table were not required because the nebulizer device used in the study is licensed and there is no requirement to collect data on the device.

Section # and Name	Description of Change	Brief Rationale
Section 2.1 Background	Updated to include a description of the three mechanisms of action of RESP301 in fighting virus infection, including addition of new figure (Figure 2–1).	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 2.2.2 Benefit Assessment	Clarification added regarding choice of nebulizer for this study.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 3 Objectives and Endpoints	Safety endpoint for AEs amended to include both counts and cumulative incidence.	Both types of data will be summarized.
Section 3 Objectives and Endpoints, Section 9.4.4 Tertiary/Exploratory Endpoint(s)	Exploratory endpoint: timepoint for change from baseline measurement of fraction of inspired oxygen (FiO_2) changed from Day 7 to Day 10.	Correction; the endpoint should be measured at end of treatment.
Section 4.4 End of Study Definition	The following clarification was added to the definition: "End of the study is defined as the last participant's last visit or follow up call."	Clarification of definition.

Section # and Name	Description of Change	Brief Rationale
Section 6.1 Study Intervention(s) Administered	This section (including Table 6-1) was updated to include dosing instructions for the first 10 participants (ie, up to the first IDMC safety review). In the event of bronchospasm, rescue bronchodilator can be initiated if needed. A new figure was also added to clearly show the staggered approach (Figure 6-1). The TID dosage was also clarified as every 8 hours with at least 6 hours between consecutive doses. Dosing Instructions were updated to clarify that nasal oxygen may be administered during nebulization if the participant is unable to tolerate nebulization without supplemental oxygen.	Specific study drug administration guidelines (including the use of a rescue medication) were provided to monitor tolerability and response to RESP301 inhalation (as described in the risk-benefit table in Section 2.2.1) and to align with the IB, Version 2.0, dated 27 May 2020.
Section 6.2.1 Preparation of Study Intervention Product	Instructions for mixing the two solutions were updated. Further clarification on the delivered dose was added.	Updated to align with the IB Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 6.5.1 Rescue Medication	A new section was added to describe allowed bronchodilator rescue medication for participants treated before the first IDMC safety review (short acting beta agonist such as salbutamol/ albuterol according to investigational site standard practice for acute bronchospasm)	Allowed rescue medication was added to minimize the risk of bronchospasm to participants, and to align with the IB Version 2.0, dated 27 May 2020.
Section 7.1 Discontinuation of Study Intervention	Rationale was added for additional monitoring and discontinuation guidance in case of bronchospasm. Table 7-1 added to provide additional clarification on the differences in signs and symptoms for acute bronchospasm and COVID-19 progression.	Safety guidance (including the use of a rescue medication) was updated to minimize the risk of bronchospasm to participants (as described in the risk-benefit table in Section 2.2.1) and to align with the IB Version 2.0, dated 27 May 2020.
Section 7.1 Discontinuation of Study Intervention Section 7.2, Temporary Discontinuation of Study Intervention Subsequent subsections of renumbered to account for new Section 7.2.	Heading updated to "Permanent" Discontinuation of Study Intervention. Text relating to temporary discontinuation moved to new Section 7.2.	Text describing temporary discontinuation now moved into separate section for clarity and consistency.

Section # and Name	Description of Change	Brief Rationale
Section 8.3.1 Time Period and Frequency for Collecting AE and SAE Information	Corrected statement that AEs and SAEs will be collected from signing of informed consent and clarified that SAEs will be reported within 24 hours of the Investigator becoming aware of the SAE, to align with Appendix 4 (previously it stated 'from randomization'). Deleted second paragraph.	Internal document consistency.
Section 8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs	Loss of sense of taste and smell added to the list of common DREs in participants with COVID-19.	Updated in line with updated World Health Guidelines.
Section 9.2 Sample Size Determination, Section 9.5 Interim Analyses, and Appendix 5, Section 10.5.1 Some General Design Considerations	The timing of the interim analysis was changed from when 60 participants have completed the Day 7 assessment to when 60 participants have completed the Day 10 assessment to make it consistent with the planned treatment duration and the SoA.	Internal document consistency.
Section 9.4.5.2 Clinical Laboratory Evaluation	The following sentence was added: Participants who had urine pregnancy test at screening and the results will be listed.	Updated to include pregnancy test data collection.

Section # and Name	Description of Change	Brief Rationale
Appendix 1, Section 10.1.3 Informed Consent Process, and Section 10.1.4 Data Protection	Text added clarifying that a separate optional ICF will be provided for US participants who agree to tokenization.	Added clarification that tokenization is for US participants only.
Appendix 4, Section 10.4.3 Recording of follow-up of AE and SAE, Section 10.4.4 Reporting of SAE	Alignment of reporting details to state 'Parexel Safety Services' throughout appendix. Text was added to include paper SAE data collection tool.	Internal document consistency and inclusion of paper SAE reporting data collection tool.
Appendix 7, Abbreviations	Heading number updated to reflect deletion of Appendix 6. Updated to reflect changes to abbreviations used in the document.	Consistency.
Section 11 References	Five new references were added to support the above updates (Basu et al, Benz et al, Brannen et al, Colosanti et al, and Saura et al). Two references were deleted as they are no longer cited.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Whole document	Minor language and format changes.	For improved clarity and readability.

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Investigator Agreement Page

Declaration of the Principal or Global Coordinating Investigator

Title: An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 requiring supplemental oxygen (NOCoV2)

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the *Declaration of Helsinki* and the guidelines on Good Clinical Practice.

Principal or Global Coordinating Investigator

Clinical Study Protocol

Protocol Title:	**An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 WHO grade 3&4 (NOCoV2)**
Short Title:	An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301+SOC vs SOC in hospitalized participants with COVID-19 WHO grade 3&4
Compound:	RESP301
Indication:	Mild to moderate COVID-19
Study Sponsor:	Thirty Respiratory Limited PPD London, W1K 6PL, United Kingdom
Protocol Number.:	RESP301-002
Study Phase:	Phase 2/Phase 3
Regulatory Agency Identifying Number:	IND No: Pending EudraCT No: 2020-002120-37
Approval Date of Current Version:	Final, 16-Sep-2020; Amendment 3 (Version 4.0)
Dates and Versions of Previous Protocols:	10 Jun 2020; Amendment 2 (Version 3.0) / 05 Jun 2020; Amendment 1 (Version 2.0) / 01 May 2020 (Version 1.0)

Sponsor Signatory:

PPD

16 September 2020

PPD

Date

Medical Monitor Name and Contact Information

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Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY		
Document	**Version**	**Date**
Amendment 3.0	Version 4.0	16-Sep-2020
Amendment 2.0	Version 3.0	10-Jun-2020
Amendment 1.0	Version 2.0	05-Jun-2020
Original Protocol	Version 1.0	01-May-2020

Amendment 3.0 (16-Sep-2020)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the EU.

Overall Rationale for the Amendment:

Three protocol changes are proposed in this substantial amendment to (a) expand the inclusion criteria to allow enrolment also of patients in hospital with COVID-19 but not yet requiring supplemental oxygen, and (b) allow a minor temporary temperature excursion in the storage conditions of the IMP to facilitate transport from the manufacturer to the clinical site or from the clinical site pharmacy to the bedside, and (c) to raise the exclusionary level for methaemoglobin to >2%.

During the peak of the first wave of COVID-19, there was a sharp influx of patients to hospitals and the vast majority of admissions were at WHO grade 4 and above – i.e. those in need of supplemental oxygen, or intensive care. More recently however, as the hospital numbers have fallen, less severely ill patients have been admitted to hospital, with a significant proportion now being at WHO grade 3. This amendment is proposed to allow the recruitment of grade 3 as well as grade 4 patients, reflective of the changing hospital admissions pattern. Treating patients earlier in the onset of the disease should help patients and reduce the burden on Intensive Therapy Units and respiratory wards.

The storage conditions for the IMP as outlined in the IMPD state that RESP301 must be stored below 25ºC (range 15-25ºC). On two occasions during transport of the IMP there has been a slight temperature excursion; the first occasion was during transport of the IMP from the manufacturer to the hospital pharmacy when the temperature rose to 25.4ºC. The second

occasion was during transport from the hospital pharmacy to the ward and the temperature rose to 25.0ºC. On both occasions the protocol conditions were followed and the manufacturer was contacted and permission given to proceed. However, on the second occasion the delay resulted in the patient not receiving the scheduled dose. The IMP has been on stability testing at 40ºC and 75% relative humidity and has remained completely stable for 3 months under these conditions. We do not wish to amend the overall storage conditions but request an amendment to allow for short-term temperature excursions up to 30ºC for less than 1 hour while the IMP is in transit.

Whereas the normal range for methaemoglobin (mHb) is <1%, this does not take account of the role of comorbidities that might affect the mHb level. Comorbid disorders that impair oxygen transport, such as cardiac and respiratory conditions, can exacerbate a low level mHb (Groeper K et a.l South Med J 2003;96:504-509). Symptomatic low-grade methaemoglobinaemia is associated with certain treatments for pneumonia, chronic obstructive pulmonary disease and heart disease (Dunford LM et al. Biol Blood Marrow Transplant 2006;12:241-242, Skold A et al. South Med J 2011;104:757-761). Since many of the patients admitted to hospital with COVID-19 may also have comorbidities that could affect the mHb level it is proposed that the exclusion level for mHb be raised to >2%.

A number of non-substantial amendments were also made and are included in the table below.

Section # and Name	Description of Change	Brief Rationale
Substantial Amendment 1		
Title; 1.1; 4.1	Change of Title to include ‘WHO grade 3 & 4;	See above
1.1 Primary Endpoint Also Section 3. 9.4.2	Previously measured progression of level 4 patients to > 4 ; now considers progression “by at least one level higher”.	To handle grade 3 entrants, as well as grade 4, to the study. See above
1.1 Secondary Endpoint Also Section 3 9.4.3	Amend secondary endpoints to measure time to change in WHO grade for those in level 3, as well as those in level 4.	See above
Throughout	Change of wording to refer to use of supplmental oxygen ‘if applicable’.	WHO grade 3 do not require supplemental oxygen, so not all study patients will be using supplemental oxygen.

Section # and Name	Description of Change	Brief Rationale
9.1 , 9.2,	Previous target was level 5 from level 4; now amended to 'at least one level' to handle the participants who enter at level 3.	
Substantial Amendment 2		
6.2.2	Addition of text to permit temperature excursions no longer than 1 hour.	See above
Substantial Amendment 3		
2.2.1 5.3, 13	Amendment of Methb threshold from 1% to 2%	See above
Non-substantial amendments		
1.3 Schedule of Activities	Changed description of oxygen measurements pre/post treatment to make clearer and remove repetition.	Instructions were repetitive and unnecessarily confusing
1.3 Schedule of Activities. Footnote 6f	Correction of 5 to 20, to match text elsewhere in document.	Correcting typo error
5.3 Point 2	Amend requirement to receive nebulization from 4 minutes to 8 minutes	4 minutes was an error; 8 mins is expected length of treatment.
6.2.1 et alia 6.4, 8.1.2	Change of 'medical supervision' to 'clinical supervision', and ;a physician' to 'the clinical team'.	As monitoring does not require a doctor.
6.2.2	Addition of text to permit temperature excursions no longer than 1 hour.	See above
7.1	Remove reference to direct bilirubin, since this is no longer to be measured.	
7.1	Remove wording 'non invasive or'	Study intervention to be discontinued only if invasive ventilation is required.
8	Maximum amount of blood updated to reflect volume and number of tests	
8.2.2	Sentence regarding vital signs removed as duplicate of previous text in the section	

Section # and Name	Description of Change	Brief Rationale
10.3	Remove unnecessary bloodtests: Bicarbonate/CO2; Serum Glutamic-Oxaloacetic Transaminase ; Serum Glutamic-Pyruvic Transaminase ; direct bilirubin	

Amendment 2.0 (10-Jun-2020)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the EU.

Overall Rationale for the Amendment:

The protocol was updated to clarify that staggered dosing is required for all participants treated before completion of the first Independent Data Monitoring Committee (IDMC) review, and to clarify exclusionary criteria and monitoring of methemoglobin (mHb). Information was added to ensure that investigators are aware of the potential risk of interaction between nitric oxide (NO) and other NO donor agents. Other changes, with brief rationale, are summarized in the following table.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 Schedule of Activities (SoA)	Footnote 4 updated to include mHb assessment at the same time points as hematology and clinical chemistry assessments.	For monitoring and assessment of mHb against the discontinuation criteria.
Section 2.2.1 Risk Assessment Section 5.3 Exclusion criteria	Table 2-1 updated to include exclusionary mHb >1%. Exclusion criterion 13 updated to specify that mHb >1% is exclusionary.	The normal reference range for mHb is 0-1% (Longo et al, 2011).

Section # and Name	Description of Change	Brief Rationale
Section 4.1 Overall Design Section 6.1 Study Intervention Administered Section 9.6 Data Monitoring Committee (DMC)	Due to the urgency of the pandemic, if participants become eligible for the study during the IDMC review of the first 10 participants then they may be recruited. However, their administration of RESP301 will be staggered in the same way as for the first 10 participants. Once the IDMC review has been completed, and provided there are no safety concerns, further recruitment will continue as per protocol. The Sponsor and contract research organization (CRO) confirm that the report from the IDMC will be sent in a timely manner to all participating sites and investigators.	The staggered dosing approach will be used for all participants enrolled up to completion of the first IDMC safety review, to ensure appropriate safety procedures are in place for the first participants recruited into the study.
Section 6.5.1 Potential Drug Interactions	A new section was added to describe NO donor agents that may increase the risk of developing methemoglobinemia.	To ensure that investigators are aware of the potential risk of interaction between NO and other NO donor agents.
Section 7.1 Permanent Discontinuation of Study Intervention	Addition of Hy's law discontinuation criteria to the first bullet.	To ensure consistency with Appendix 10.3.
Whole document	Minor language and format changes.	For improved clarity and readability.

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1 Protocol Summary

1.1 Synopsis

Protocol Title: An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 WHO grade 3 & 4 (NOCoV2)

Sponsor Protocol No.: RESP301-002

Study Phase: Phase 2/Phase 3

Sponsor: Thirty Respiratory Limited

Rationale:

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design. Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer 3 times a day (TID) for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 3 or 4 in the modified World Health Organization (WHO) ordinal scale into higher levels	• Proportion of participants who progress by at least one level higher on the modified WHO ordinal scale by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO_2	• Change in room air SpO_2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time

Objectives	Endpoints
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement of at least one level lower on the modified WHO ordinal scale • Time to progression of at least one level higher on the modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	

• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements
	• Physical examinations
	• Vital signs
	• Concomitant medications
	• Counts and cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

Overall Design:

- Open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 added to standard of care (SOC) in hospitalized patients with COVID-19 WHO ordinal scale grade 3 & 4.

- Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as detailed in Section 6.3.

- The study treatment will be permanently discontinued prior to 10 days if the participant:

    - Improves to level 1 or 2 of the modified WHO ordinal scale;
    - Progresses to a level > 4 of the modified WHO ordinal scale;
    - Experiences an event that requires permanent discontinuation as described in Section 7.1.

- An Independent Data Monitoring Committee (IDMC) will be responsible for closely reviewing the safety and efficacy data from interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

- A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). Each RESP301 dose will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6).

Disclosure Statement: This is a parallel group treatment study with two arms that are open-label.

Number of Participants:

Approximately 300 adult (male or female) participants will be randomly assigned to study intervention (200 to the Investigational arm and 100 to the Control arm).

Intervention Groups and Duration:

The total study duration for a participant from screening to last follow up will be up to 30 days (±2 days). The study will be divided into the following periods:

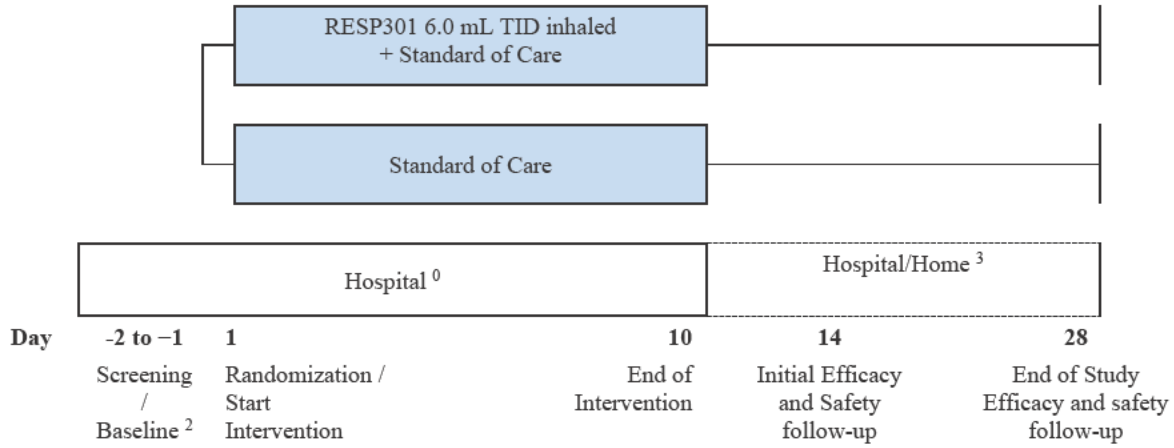
- Screening period: starts up to 2 days (48 hours) prior to and extends up to the day of treatment initiation (Day 1); Days −2, −1 and 1
- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28 (both ±2 days)
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±2 days)

Participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a nebulizer TID for up to 10 days in addition to the SOC, while participants in the Control arm will receive SOC alone.

Data Monitoring Committee: Yes

1.2 Schema

Figure 1–1: Study Design



TID=3 times daily

1 Participants may be discharged from hospital before Day 10.

2 Screening period may include Day 1, as participants may be screened and randomized on the same day provided all eligibility criteria are met and the participant had sufficient time to consider their participation in the study.

3 The post-treatment efficacy and safety follow-ups may be conducted by telephone for participants who are discharged from the hospital at the time.

1.3 Schedule of Activities (SoA)

Table 1-1 Schedule of Activities (SoA)

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Informed consent	X							Optional tokenization ICF to be provided for a subset of participants in the United States (see Section 8.4)
Confirmation of COVID-19 infection by nasopharyngeal RT-PCR [3]	X							
Inclusion and exclusion criteria	X	X						Recheck clinical status before 1st dose of study intervention
Demographics	X							
Physical examination including height and weight	X			X	X		X [2]	Full physical exam and height only at screening

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Pregnancy test (urine)	X							All women, including women of childbearing potential and pregnant women, may be enrolled in the study. Pregnancy status should be recorded (see Section 8.3.5). Note: Only females of child bearing potential and pregnant females need to undergo a screening pregnancy test.
Medical history (includes substance usage)	X							History of substance usage or abuse to be recorded but is not exclusionary
Medication history	X							In the prior 3 months
Laboratory assessments (including liver chemistries) [4]	X [4]	X [4]	X (Days 3 and 7 only) [4]	X [4]	X [4]		X [2,4]	
12-lead ECG	X							After resting supine for approximately 5 minutes. QRS, QT, QTcF, PR, RR, rate
Vital signs [5]	X	X	X [5]	X	X		X	Within 15 minutes prior to study intervention administration

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Randomization		X						
Study intervention, TID [6]		X	X	X				A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). Each RESP301 dose will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6). Where possible study intervention should be administered at approximately the same times each day At least 6 hours between 2 consecutive doses
AE/SAE review	X	X	X	X	X	X	X	
Concomitant medication review	X	X	X	X	X	X	X	
Pre Treatment oxygen / respiratory assessment [7]	X	X	X	X				
SpO_2 on room air (after at least 1 minute) prior to study intervention administration and immediately at the end of nebulization [6]		X	X	X				See Section 8.1.2

Procedure	Screening (up to 48 hours before Day 1 [1])	Intervention Period			Early withdrawal	Efficacy follow-up (Day 14) [2] (± 2 days)	EoS efficacy and safety follow-up (Day 28) [2] (± 2 days)	Notes
		Day 1 [1]	Daily while hospitalized	Day 10 / hospital discharge				
Modified WHO ordinal scale	X	X	X	X		X	X	See Section 8.1.1
NEWS 2 assessment [7]		X	X	X				Prior to study intervention in the morning. See Section 8.1.3

Abbreviations: AE=adverse event; aPTT= activated partial thromboplastin time; CBC=complete blood count; Chem 7=blood chemistry panel (sodium, potassium, chloride, bicarbonate/CO_2, blood urea nitrogen, creatinine, glucose); CV=cardiovascular; ECG=electrocardiogram; IDMC=Independent Data Monitoring Committee; LFTs=liver function tests; mHb=methemoglobin; PT/INR=prothrombin time/international normalized ratio; RT-PCR=reverse transcription-polymerase chain reaction; SAE=serious adverse event; TID=three times daily.

1. Screening and randomization may be on the same day, providing all eligibility criteria are met.
2. For participants who are already discharged at either the Day 14 or Day 28 (EoS) follow-up, there will be a phone call to check AEs/SAEs, concomitant medications, and modified WHO ordinal scale. At EoS, safety laboratory tests (nitrite and mHb only) and weight will be collected for participants in hospital only.
3. COVID-19 infection must be confirmed and documented in chart with a positive result on a validated test.
4. At Screening: CBC, chem 7, mHb, LFTs, and coagulation (PT/INR, aPTT).

   On Day 1, Day 3, Day 7 and Day 10, or early withdrawal: CBC, chem 7 (per SOC), and mHb.

   On Day 28: nitrite and mHb (participants in hospital at EoS only).
5. Vital signs: temperature, heart rate, blood pressure, respiratory rate. Vital signs to be repeated as per SOC during treatment period.
6. Study intervention comprises the following procedures which are to be done in order and as quickly as possible:
   a. Discontinuation of supplemental oxygen administration where applicable *
   b. Close participant oversight by trained health care professional until SpO_2 is stable on ambient air after one minute at least *
   c. Recording of SpO_2 * pre-nebulization on ambient air
   d. Preparation of the admixture of RESP301 (to be done no longer than 5 minutes before administration)*
   e. Administration of the nebulization via a vibrating mesh nebulizer while monitoring SpO_2 and heart rate (alarms set to 89% and 120 beats / min, or as indicated by the Investigator, for stopping study intervention administration and resuming supplemental oxygen where applicable) * If the participant is unable to tolerate nebulization without supplemental oxygen, nasal oxygen may be administered during nebulization.
   f. Discontinuation of nebulization 20 minutes after preparation of admixture, or no product left in the device, whichever comes first *
   g. Recording of SpO_2 * post nebulization on ambient air
   h. Resuming supplemental oxygen where applicable*
   i. Participant oversight until SpO_2 is stable *
   j. Recording of SpO_2 *

   * *for participants on active treatment arm only*
7. Participant remains on supplemental oxygen if applicable,and measurements made of respiratory rate, heart rate and SpO_2 and a record of of oxygen flow level including mode (nasal cannula, mask)

2 Introduction

2.1 Background

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by a novel coronavirus (SARS-CoV-2). COVID-19 was first described in Wuhan, China, in December 2019 and is now a global pandemic (Matos et al, 2020). Most of those affected have milder illness (80%), 15% will be severely ill (require oxygen) and 5% will require intensive care unit care (Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Of those who are critically ill, most require early intubation and mechanical ventilation. Other complications include septic shock and multi-organ failure, including acute kidney injury and cardiac injury (Yang et al, 2020). Older age and comorbid diseases, such as chronic obstructive pulmonary disease (COPD), hypertension, and diabetes, increase risk of death (Huang et al, 2020; Zhou et al, 2020). The virus is highly contagious and spread via respiratory droplets, direct contact and, if aerosolized, airborne routes. The most common symptoms include fever, fatigue, dry cough, and shortness of breath.

A critical early component of innate host defense in the airway is the ability of respiratory epithelial cells to produce high levels of NO (Kao et al, 2001). NO functions as a signaling molecule in initiation of the inflammatory response to viruses, and also has direct antiviral effects (Folkerts et al, 1998). The airway epithelium has highly efficient nitric oxide (NO) synthetic machinery which is amplified in viral infection. Healthy human airway epithelium has abundant expression of the endothelial enzyme NOS II due to continuous transcriptional activation of the gene in vivo. Loss of NO synthesis in lung diseases predisposes individuals to increased virus/microbe infection (Xu et al, 2006).

The physiological roles of NO and the enzymatic pathways for its synthesis via NO synthase have been clearly established for many years (Tucker et al, 2007). In particular, NO has been demonstrated to have potent anti-microbial properties against a wide range of pathogens. An alternative non-enzymatic synthetic pathway for NO synthesis has been developed, which generates NO and related higher oxides of nitrogen (NOx) via the chemical reactions of acidified nitrite (Hardwick et al, 2001, Tucker et al, 1999). A solution of co-mixed NO/NOx and ascorbic acid (RespiNOS), delivered by nebulizer, was found to be safe and well tolerated in healthy volunteers (Tucker et al, 2007). Spectral investigations further confirmed that there were no potentially harmful moieties present in the solution. The study concluded that RespiNOS had potential for use as a broad-spectrum anti-microbial agent in patients with chronic bronchial sepsis such as bronchiectasis, COPD, and cystic fibrosis.

RESP301 is a NO-generating liquid designed to release NO in situ in the upper airways and deep in the alveolar spaces. RESP301 is an admixture solution of two precursor solutions mixed together at point of care for immediate inhaled administration via a CE-marked handheld nebulizer and has specific advantages (see below) over inhaled NO gas in treating patients with COVID-19 during the current pandemic. It has shown high in vitro activity against respiratory pathogens, both viral and bacterial.

Key advantages of RESP301 over inhaled NO gas are:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

The Sponsor has conducted a number of supportive in vitro studies to assess the activity of NO-generating solutions against major respiratory viruses responsible for infections in humans. These studies include the following:

- In vitro studies to demonstrate the effects of RESP301 on SARS-CoV-2 replication
- In vitro study of RESP301 against influenza A (H1N1) and human rhinovirus
- Determination of the anti-viral efficacy of NO-releasing formulations against two strains of virus (influenza A virus [H1N1] and human rhinovirus 16)
- Anti-microbial activity of acidified nitrite solutions against intracellular drug sensitive and drug resistant *Mycobacterium tuberculosis* and *M. abscessus* infections
- Anti-microbial activity of RESP301 when used alone and in combination with antibiotics

Recent data from experiments at two independent laboratories using two different isolates of SARS-CoV-2 suggest that incubation with RESP301 for 36 to 48 hours reduced viral load to levels below the limit of detection of the assay at concentrations which were not associated with cytotoxicity. The limiting factor of the experiments was the slow growth rate of the viral controls; reduction in viral load was 2.2 and 4 Log_{10} median tissue culture infectious dose ($TCID_{50}$).

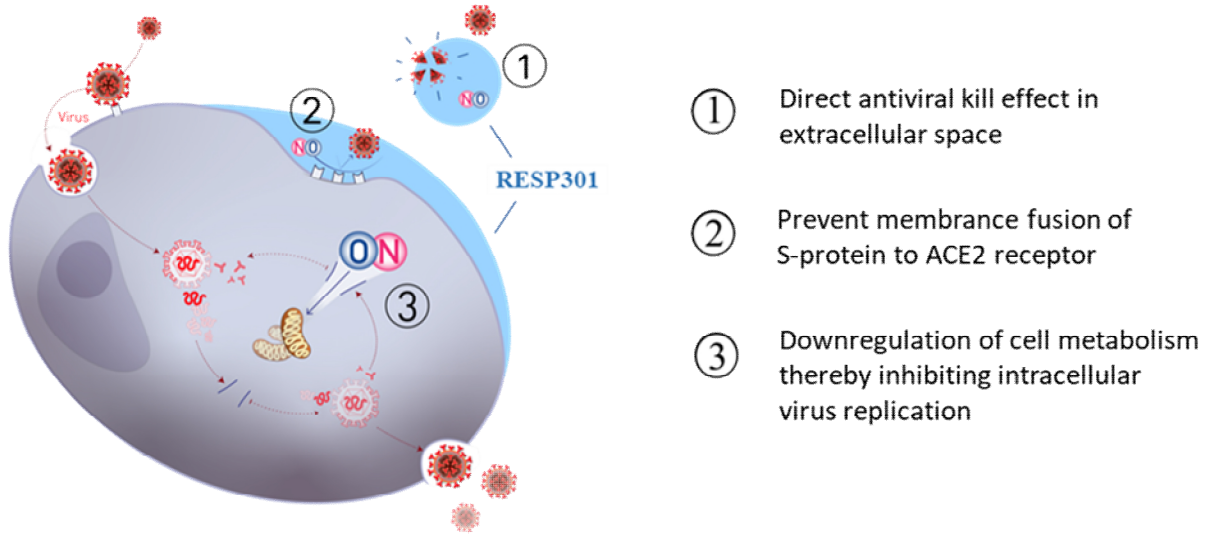
Studies of inhaled nebulized sodium nitrite (AIR001) in healthy subjects and in patients showed that inhaled acidified nitrite produces dose proportional plasma pharmacokinetics without accumulation following repeated administration, with low systemic blood levels of nitrite and

methemoglobin (<3%) (Rix et al, 2015). Inhaled nitrite (AIR001) at doses up to 90 mg three times daily (TID) displayed a good safety profile and is well tolerated (Parsley et al, 2013; Simon et al, 2016), thus supporting the investigation of RESP301 in the clinical setting.

RESP301 delivers NO in a specifically focused way and with a unique mode of administration. It is an important evolutionary step in delivering NO that is more physiological and overcomes the technical difficulties and efficacy limitations of current NO gas therapy. Whereas inhaled NO gas has been used to treat patients with virus-induced acute respiratory distress syndrome and is being tested currently in COVID-19 patients, it focuses on increasing vascular permeability and is not sufficiently targeted at the infection, it is rapidly dispelled with exhalation and is oxidized in air to toxic NO_2. By comparison, RESP301 generates NO in a liquid environment that comes in direct contact with the virus and the virus-infected cells. As a result, RESP301 has a better targeted approach, and lower concentrations of NO are required, with a much more portable delivery system. An important environmental safety factor is that RESP301 produces negligible levels of NO_2 generated and exhaled NO.

In generating NO, RESP301 has at least three distinct mechanisms of action in fighting virus infection, two of which are common to many viruses and one is very specific to coronaviruses, such as SARS-CoV-2 (Figure 2–1).

Figure 2–1: Mode of Action of RESP301 Against SARS-CoV-2



1. Nitric oxide has a direct kill effect on the virus in the extracellular space. Nitric oxide-mediated nitrosylation of viral and host macromolecules appears to block viral replication and this has been demonstrated for several viruses (Saura et al, 1999; Basu et al, 1999). Enzymes, such as proteases (reverse transcriptases, and ribonucleotide reductase), vital for the life-cycle of the virus, are targets for NO nitrosylation (Benz et al, 2002; Colosanti et al, 1999).
2. In coronaviruses like SARS-CoV-2, NO specifically prevents membrane fusion of the S-protein (the spike protein that gives the virus its characteristic crown-like appearance) to the angiotensin-converting enzyme 2 receptor on the host cell.
3. Nitric oxide can attack the virus indirectly. Since viruses are non-living entities and do not have their own metabolism, they hijack the host cell's metabolic processes, and derive their energy and specific cellular substrates from the host cell. Nitric oxide suppresses the virus-induced hyperactivity in the host cell and thereby deprives the virus of its crucial supply chain.

RESP301 efficiently delivers with all three modes of action and is therefore considered an ideal candidate for clinical trials in COVID-19.

A detailed description of the chemistry, pharmacology, efficacy, and safety of RESP301 is provided in the Investigator's Brochure.

2.2 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of RESP301 may be found in the Investigator's Brochure.

2.2.1 Risk Assessment

Table 2-1 Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention RESP301		
Participants may need to be discontinued from supplemental oxygen for	Study treatment is to be administered via a vibrating mesh nebulizer which is designed for oral inhalation.	Participants unable to be safely discontinued from supplemental oxygen will be

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
several minutes to receive nebulized study treatment		excluded (Exclusion criterion 2) Treatment administration is to be performed as quickly as possible (Section 1.3) Oxygen saturation (SpO_2) and heart rate will be monitored throughout the study intervention administration (Section 1.3) In case of decrease in SpO_2 or clinical signs of intolerance to nebulization, RESP301 treatment may be paused temporarily and supplemental O_2 resumed or the study treatment may be discontinued permanently (see Section 7.1) per Investigator assessment.
Risk of methemoglobinemia	Very low levels are produced but as methemoglobinemia has been reported with continuous NO gas administration, the risk cannot be firmly ruled out	Participants with a history of methemoglobinemia will be excluded (Exclusion criterion 4), methemoglobin (mHb) level >2% is exclusionary (exclusion criterion 13), and mHb is included in safety laboratory tests (Section 1.3)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Risk of clinical bronchial hyper-responsiveness related to nebulization	Excipients in the nebulized RESP301 include mannitol which is a known bronchial irritant at the average dose of a positive mannitol test (239.0 ± 185.0 mg, Brannan et al, 2005). While the dose used in this study (54.66 mg) is lower than the average dose for a positive mannitol test, it has not yet been tested in COVID-19 patients and may potentially cause or exacerbate cough or bronchospasm in susceptible individuals in this specific population	Participants with a known history of moderate or severe bronchial hyperreactivity (such as in asthma) or presence of signs of significant bronchospasm on examination will be excluded from study participation (Exclusion criterion 5) For the first 10 participants treated before the first Independent Data Monitoring Committee (IDMC) safety review (Section 9.6), RESP301 will be administered by intermittent inhalation until the intended dose is delivered (Section 6.1), so that the treatment can be quickly adjusted and rescue bronchodilator initiated if needed (Section 6.5.2) After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by continuous inhalation.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Nebulization	The delivered dose of RESP301, estimated to be ~40% of the masses listed in Table 6-2, can be affected by patient technique and inspiratory capacity leading to inter- and intra-patient variation in actual doses of these ingredients, given the acute nature and high disease burden in this proposed clinical setting	Training will be provided to study staff to minimize variation in dose
Other		
Risk of spreading SARS-CoV-2	SARS-CoV-2 is known to be transmissible via respiratory droplets and any interventions that may potentially increase cough or aerosolization of respiratory secretions of an infected patient may increase risk of spread of disease	Proper personal protective equipment including appropriate mask, face shield, gown and gloves should be used at all times. When possible, the site staff should not remain in the room during study intervention administration (providing that continuous O_2 monitoring can be conducted remotely and study intervention can be administered correctly)

2.2.2 Benefit Assessment

To date, no treatment of COVID-19 has demonstrated clinical efficacy. Patients and their physicians are critically in need for treatments that decrease the risk of severe levels of disease,

particularly the rate of intubation or other ventilatory support as they significantly lead to fatal outcomes. Considering the current pandemic, even a limited improvement in the rate of progression to severe stage of the disease would provide sizable benefits for patients and society.

Nitric Oxide is already marketed (e.g., INOmax®, NOXIVENT™, etc) in the United States (US) and other countries as a gas for continuous use in preterm and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension in conjunction with ventilatory support and other appropriate agents. It is well tolerated, although under continuous use, a small number of cases of methemoglobinemia have been reported (NOXIVENT™ FDA Prescribing Information).

Nitric oxide has been shown in vitro to inhibit the replication cycle of severe acute respiratory syndrome coronavirus (Akerström S et al, 2005). In addition, NO is a naturally occurring and potent antimicrobial agent in the human body, which is active against viruses, bacteria, fungus, and yeasts (Fang, 1997). In particular, NO inhibits replication in vitro in a number of respiratory viruses including influenza A and B (Rimmelzwaan et al, 1999, Regev-Shoshani et al, 2013), human rhinovirus (Sanders et al, 1998), and respiratory syncytial virus (Ali-Ahmad D et al, 2003).

The ability of RESP301 to have a marked antibacterial action is highly relevant in the context of treating patients with SARS-CoV-2 infection since superimposed bacterial infection is a critical factor and a major cause of morbidity and mortality. RESP301 would restore and replenish the NO deficiency in patients who have succumbed to SARS-CoV-2 infection. RESP301 also has key advantages over inhaled NO gas, as discussed in Section 2.1.

A vibrating mesh nebulizer is advised for administration of RESP301. Amongst the suitable nebulizers, the device chosen for the study is a commercially available, CE-marked vibrating mesh device that delivers a mist of fine droplets in the size range required for pulmonary deposition (Mass Median Aerodynamic Diameter < 5 μm). This has been shown to be ideally suited for penetration deep into the alveolar spaces of the lung. As with all nebulizers in clinical use, there is a residual amount of drug that remains in the nebulizer so that not the full amount of drug is administered. The device operates continuously once initiated and automatically switch off once the medication has been delivered. The device may be used in pediatric and adult populations, as permitted by the prescribed medication, and is suitable for use in home environments or hospital/clinic settings.

As testing inefficient product would distract sites from participating in other research efforts, two interim analysis for futility purpose have been planned.

2.2.3 Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to participants in this first-in-man study, the potential risks identified in association with RESP301 are justified by the anticipated benefits that may be afforded to participants with COVID-19.

3 Objectives and Endpoints

Table 3-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate efficacy of RESP301 in preventing progression of hospitalized COVID-19 participants at level 3 or 4 in the modified World Health Organization (WHO) ordinal scale into higher levels.	• Proportion of participants who progress by at least one level higher on the modified WHO ordinal scale by Day 14
Key Secondary	
• To assess the effect of RESP301 as measured by room air SpO_2	• Change in room air SpO_2 from baseline over time
• To assess the effect of RESP301 as measured by the National Early Warning Score (NEWS) 2 symptom score	• Change in NEWS 2 symptom score from baseline over time
• To assess the treatment response on clinical status	• Change from baseline on the modified WHO ordinal scale at each visit up to Day 28 • Time to improvement of at least one level lower on the modified WHO ordinal scale • Time to progression of at least one level higher on the modified WHO ordinal scale
Additional Secondary	
• To assess the treatment response on clinical status	• Time to hospital discharge • Incidence of mortality by Day 28
Safety	
• To assess the overall safety profile of RESP301 in COVID-19 participants	• Clinical safety laboratory measurements • Physical examinations

Objectives	Endpoints

	• Vital signs
	• Concomitant medications
	• Counts and cumulative incidence of:
	  ○ Adverse events (AEs)
	  ○ Serious adverse events (SAEs)
	  ○ Severe AEs

• To assess the ability of participants to tolerate nebulization	• Incidence of participants unable to tolerate nebulization due to:
	  ○ Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
	  ○ Other clinical signs of intolerance according to Investigator's opinion
	• Incidence of clinical bronchial hyper-responsiveness related to nebulization

Exploratory	
• CCI [redacted]	• CCI [redacted]

4 Study Design

4.1 Overall Design

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study in hospitalized participants with COVID-19 (WHO ordinal scale level 3 or 4) using a sequential adaptive design to evaluate the efficacy and safety of RESP301 added to standard of care (SOC). Approximately 300 adult (male and female) participants will be consented and randomized 2:1 to the Investigational arm or the Control arm. Participants randomized to the Investigational arm will receive inhaled RESP301 (6 mL) administered using a vibrating mesh nebulizer TID for up to 10 days in addition to the standard of care (SOC) while participants in the Control arm will receive SOC alone.

The study will be divided into the following periods:

- Screening period: starts up to 2 days (48 hours) prior to and extends up to the day of treatment initiation (Day 1); Days −2, −1 and 1

- Intervention period: up to 10 days; Day 1 to Day 10
- Efficacy follow-up: Day 14 and Day 28 (±2 days)
- Safety follow-up: 28 days after treatment initiation; with the participant being contacted by phone call, if not still hospitalized, on Day 28 (±2 days)

After screening, eligible participants will be randomized to either RESP301+SOC or SOC alone on Day 1, and the study treatment will be initiated as applicable (Table 1-1). Screening and randomization may happen on the same day (Day 1).

An individual administration of study intervention will be stopped, and supplemental oxygen resumed if applicable, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Section 4.2 and Section 8.1.2). However, the participant may continue with subsequent doses if the Investigator judges that the benefit / risk ratio remains positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored. Inhalation of study intervention should be completed within 20 minutes. A staggered dosing approach will be used for the first 10 participants (and enrolment up to completion of the first IDMC safety review) to monitor tolerability and response to RESP301 (see Sections 6.1 and 9.6).

The study treatment will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale;
- Experiences an event that requires permanent discontinuation as described in Section 7.1.

In the above cases, participants will be considered as ongoing in the study until the final Safety follow-up phone call (Day 28). Participants will be withdrawn from the study prior to Day 10 (Early withdrawal) only if they withdraw consent.

The total study duration for a participant from screening to last follow up will be up to 30 days (±2 days).

Participants will be stratified by country and presence of risk factor(s) for severe outcomes of COVID-19, based on comorbidities and age, as detailed in Section 6.3.

Two interim analyses are planned (see Section 9.5 for details).

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet after 10, 20, 60 and 150 participants have completed the study (see Sections 9.5 and 9.6).

4.2 Scientific Rationale for Study Design

NO is a naturally occurring and potent antimicrobial agent in the human body which has been shown in vitro to inhibit replication of severe acute respiratory syndrome coronavirus (Akerström S et al, 2005), including SARS-CoV2, and other respiratory viruses. NO as an inhaled gas is already marketed in the US and other countries (see Section 2.2.2) but has disadvantages over NO produced locally in the oropharynx or lung airways (lengthy treatment requiring NO canisters, the inhaled NO is expelled in exhalation, the NO gas oxidizes in air to form toxic NO_2 which is a potential lung irritant and a contaminant in the patient's environment, and the half-life of NO in air is short).

RESP301 has potential advantages over inhaled NO gas:

- RESP301 demonstrates powerful evidence in vitro as a broad-spectrum anti-microbial;
- Treatment is quick, easy and portable using a standard handheld nebulizer;
- The formulation is nebulized and forms a fine particle mist that settles on the lung surface, at the point of infection, and is therefore a more targeted approach;
- NO production continues in-situ post treatment;
- Because of the design of the formulation, production of NO_2 is negligible by virtue of the NO_2 being rapidly converted to a NO donor species.

In this study, the effect of RESP301 as an add on treatment to SOC will be evaluated for its efficacy in reducing rate of progression to a more severe level of COVID-19 and for safety by comparison with SOC alone in hospitalized COVID-19 patients. A sequential adaptive design was chosen in order to assess futility and sample size after interim analyses (see Section 9.5). The study design was developed in accordance with the latest WHO and regional regulatory agencies guidelines.

Several risks factors for severe or fatal outcomes have been reported (Bialek et al, 2020; Huang et al, 2020; Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020; Rong-Hui et al, 2020; Zhou et al, 2020). To minimize bias, the results will be stratified according to the known risk factors as described in Section 6.3.

In this study, measures will be in place to trigger discontinuation of study intervention at a SpO_2 limit pre-agreed at the site (see Section 8.1.2). There is no critical level of oxygen saturation below which tissue hypoxia occurs due to the large number of variables that contribute to

hypoxia at the tissue and cellular level (temperature, pH, tissue blood flow). As a result, there is no consensus about what constitutes normal and abnormal oximetry (ATS/ACCP, 2003). To support and guide the team in charge of study intervention administration, a default value of SpO_2=89% and heart rate (120 beats/min) has been proposed as a suitable limit of tolerance. However, for the reason stated above, this limit can be overruled by the Investigator in either direction.

Newly released guidelines for ongoing clinical trials during the COVID-19 pandemic have emphasized the need to reduce the burden on sites and clinical trial personnel/investigators whether they be administrative, site visit, or other burdens (ACRO, 2020; EMA, 2020; HRS, 2020). Thus, without blinding and a placebo, along with minimal procedures, the additional burden on clinical staff has been reduced as much as possible.

4.3 Justification for Dose

Studies with nebulized sodium nitrite (AIR001) in healthy subjects and in patients have shown that inhaled nitrite produces dose proportional plasma pharmacokinetic without accumulation following repeated administration, with low systemic blood levels of nitrite and methemoglobin (<3%). Inhaled nitrite at doses up to 90 mg TID displayed a good safety profile and is well tolerated (Rix et al, 2015; Parsley et al, 2013; Simon et al, 2016).

In this study, 6.0 mL RESP301 (delivered dose 62 mg) will be administered via a vibrating mesh nebulizer, TID with at least 6 hours between each dose. Each 6.0 mL dose for nebulization contains the masses described in Table 6-2.

4.4 End of Study Definition

A participant is considered to have completed the study if he/she has completed the Treatment Period and the Follow-up Period (through the Day 28 EoS Follow-up). End of the study is defined as the last participant's last visit or follow up call.

5 Study Population

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

The study population will consist of male and female COVID-19 patients, including pregnant women and women of child bearing potential. Participants must be able to provide written consent and meet all the inclusion criteria and none of the exclusion criteria.

5.1 Study Rationale

This is an open-label, randomized, multicenter, parallel group, concurrent, controlled study using a sequential adaptive design to evaluate the efficacy and safety of RESP301 plus SOC versus SOC alone in hospitalized patients with COVID-19 (WHO level 3 & 4).

Each constituent (see Section 6.2) of RESP301 is used as SOC for various conditions. Product application is straightforward, requiring a few minutes of inhalation using a standard nebulizer. RESP301 is likely to be beneficial in treating patients with COVID-19 who are not receiving ventilation but may be using supplementary oxygen in order to maintain a safe level of SpO_2.

Considering the well-established and global use of NO and the device, and the current public health emergency resulting from the COVID-19 pandemic, a Phase2/3 study of RESP301 is considered reasonable in order to generate efficacy data in patients infected with COVID-19.

5.2 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant is ≥18 years of age, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participant has laboratory-confirmed SARS-CoV-2 infection as determined by reverse transcriptase polymerase chain reaction (RT-PCR) or other approved clinical testing prior to randomization.
3. Participant is hospitalized in relation to COVID-19 (WHO level 3 & 4).

Sex

4. Participant is male or female. All females of childbearing potential, including pregnant females, must consent to urine pregnancy testing at screening to be eligible for the study. (Females who are not of childbearing potential do not need to undergo a pregnancy test at screening).

Informed Consent

5. Participant is capable of giving signed informed consent as described in Appendix 1 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.3 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Rapidly deteriorating or likely to require escalation to high flow oxygen, invasive or non-invasive ventilatory support within 24 hours according to Investigator's opinion.
2. Unable to safely receive a nebulized treatment for approximately 8 minutes according to Investigator's opinion.
3. Unable to receive or considered ineligible for invasive or non-invasive ventilatory support.
4. History of methemoglobinemia.
5. Uncontrolled asthma or history of severe bronchospasm.
6. Severe (requiring baseline oxygen therapy > 12 h/day prehospitalization) chronic respiratory disease (e.g., known COPD, pulmonary arterial hypertension, idiopathic pulmonary fibrosis, interstitial lung disease).
7. Suspected or confirmed untreated, active tuberculosis.
8. Severely immune-compromised participants in Investigator's opinion.
9. Recent (within 3 months) active coronary artery disease or decompensated heart failure (New York Heart Association class 3-4).
10. Presence of tracheostomy.

Prior/Concomitant Therapy

11. Chronic (≥4 weeks) use of corticosteroids >10 mg/day of prednisone or equivalent within 4 weeks of randomization.

Prior/Concurrent Clinical Study Experience

12. Participation in other clinical investigations utilizing investigational treatment or within 30 days / 5 half-lives whichever is longer.

Diagnostic Assessments

13. Clinically significant abnormalities in clinical chemistry or hematology at screening, defined as:

- Methemoglobin level >2%
- Platelet count <50,000 mm^3
- Alanine aminotransferase or aspartate aminotransferase >5 × upper limit of normal (ULN).
- Estimated glomerular filtration rate <30 mL/min/1.73 m^2 (modification of diet in renal disease formula) or requiring hemofiltration or dialysis.

Other Exclusions

14. Anticipated transfer to another hospital which is not a study site during the treatment period.
15. Allergy to any of the components of the study intervention.

5.4 Lifestyle Considerations

No restrictions are required.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography and reason for screen failure (e.g., eligibility requirements failed).

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

5.5.1 Screening and Enrollment Log and Participant Identification Numbers

The participant's enrollment will be recorded in the Screening and Enrollment Log.

Upon enrollment, each participant will receive a unique participant identification number. Participant numbers must not be re-used for different participants.

6 Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1 Study Intervention Administered

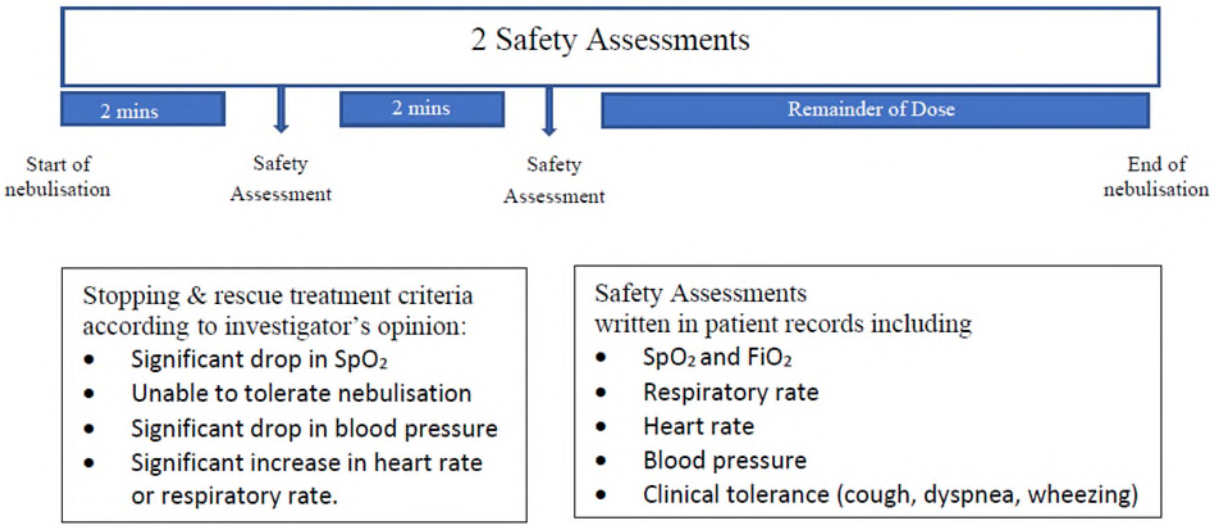
Table 6-1 Study Intervention(s) Administered

Study Treatment	RESP301 + SOC
Intervention Name:	RESP301
Type:	Drug
Dosage Formulation:	Admixture for inhalation
Unit Dose Strength(s):	Delivered dose (62 mg) sodium nitrite ($NaNO_2$) (6 mL of NO-generating admixture of 150 mM $NaNO_2$, 50 mM mannitol and 100 mM citric acid)
Dosage Level(s):	One 6 mL-inhalation TID (every 8 hours) with at least 6 hours between 2 consecutive doses.
Route of Administration:	Inhalation
IMP and NIMP:	IMP
Sourcing:	RESP301 provided centrally by the Sponsor.
Dosing Instructions:	A vibrating mesh nebulizer will be used to administer the RESP301, under the direction of the study physician. Treatment is to commence within 5 minutes of RESP301 preparation. For the first 10 participants (and enrolment up to completion of the first IDMC safety review) RESP301 will be administered by intermittent inhalation to monitor tolerability and response to RESP301. After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by a continuous inhalation. Before initiating each RESP301 inhalation, supplemental oxygen, if being used, will be interrupted for a few minutes until SpO_2 levels are stable. Depending on the extent of SpO_2 decrease, the RESP301 inhalation may be initiated or supplemental oxygen may be resumed at the discretion of the investigator. After treatment initiation, in case of SpO_2 decrease, RESP301 inhalation may be paused and supplemental oxygen resumed. If the participant is unable to tolerate nebulization without supplemental oxygen, nasal oxygen may be administered during nebulization.
Packaging and Labeling:	Study intervention will be provided as two separate vials: $NaNO_2$/mannitol (3 mL) and citric acid buffered to pH 5.4 (3 mL), which will be labeled as required per country requirement.

Staggered dosing approach for the first 10 participants:

For the first 10 participants treated, and enrolment up to completion of the first IDMC safety review (Section 9.6), RESP301 dose will be administered in a staggered approach: after 2 minutes of inhalation, treatment is briefly paused to assess the participant. Provided there is no evidence of bronchospasm, treatment is continued for a further 2 minutes, after which treatment is again briefly paused for participant assessment. Thereafter, provided there is no evidence of bronchospasm and the participant is able to continue, the dose is completed (Figure 6-1), so that treatment can be quickly adjusted and rescue bronchodilator initiated if needed (Section 6.5.2). After reviewing safety data, the IDMC will decide whether the intermittent inhalation of RESP301 can be replaced by a continuous inhalation (see also Section 9.6). Due to the urgency of the pandemic, if participants become eligible for the study during the IDMC review of the first 10 participants then they may be recruited; however, their administration of RESP301 will be staggered in the same way as for the first 10 participants.

Figure 6-1: Staggered Dosing Approach for the First 10 Participants



6.1.1 Medical Devices

1. Medical device (not manufactured by or for Thirty Respiratory Limited) provided for use in this study will be an FDA-approved and CE-marked vibrating mesh nebulizer.
2. Instructions for medical device use will be provided in the User Manual.

6.1.2 Device Training

Training will be provided to ensure all study staff are familiar with the device, including mixing the solutions, adding the admixture to the device and cleaning the device.

6.2 Preparation, Handling, Storage, and Accountability

6.2.1 Preparation of Study Intervention Product

RESP301 is prepared at bedside by mixing two sterile 3 mL solutions (one of $NaNO_2$/mannitol and one of citric acid buffered to pH 5.4). The solution is to be nebulized immediately or within 5 minutes post mixing as per the instructions given in the SoA (Table 1-1) and, for the first 10 participants, as in Section 6.1 above (staggered dosing). The first administration of study intervention should be provided under clinical supervision.

RESP301 is mixed in the nebulizer chamber before use following the steps below (see Table 6-2 for details of composition):

1. First the vial marked A ($NaNO_2$ and mannitol) is opened and its contents are poured into the nebulizer chamber.
2. Next, the vial marked B (citric acid) is opened and its contents are poured into the nebulizer chamber.
3. The nebulizer lid should be closed, and the nebulizer should be swirled gently for a few seconds to mix the two solutions. The nebulizer should not be inverted.

The inhalation of the medication should start within 5 minutes of mixing the solutions and the inhalation process should be complete within 20 minutes of starting inhalation.

This combined mildly acidified nitrite solution (6 mL) is inhaled through the mouth using a vibrating mesh nebulizer under the direction of the clinical team. The purpose of the acidified nitrite aerosol is to deliver NO to the participant. The delivered dose of RESP301, assessed via simulated tidal breathing is estimated to be ~40% of the masses listed below; it should be noted that delivered dose can be further affected by participant's breathing pattern and inspiratory capacity and so inter- and intra-participant variation in actual doses of these ingredients may vary, given the acute nature and high disease burden in this proposed clinical setting.

Table 6-2 Composition of Drug Product, Diluent and Admixture

	Material	Function	Quantity per vial
Drug Product	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Diluent	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**3.0 mL**
Acidified Drug Product Mixture (RESP301)	Sodium Nitrite	Drug Substance	62.10 mg
	Mannitol	Excipient	54.66 mg
	Citric Acid	Diluent	115.28 mg
	Sterile water for injection	Diluent	
	Total Volume		**6.0 mL**

6.2.2 Storage and Accountability of Study Intervention

The Investigator or designee must document whether appropriate temperature conditions (<25°C) have been maintained during storage for all study intervention received and any discrepancies are reported and resolved before use of the study intervention. Temperature excursions are permitted up to 30ºC for no longer than 1 hour. Such a temperature excursion must be recorded, however the study intervention can be safely administered to participants.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (e.g., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study intervention are provided in the Investigator Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an interactive voice response system (IVRS)/interactive web response system (IWRS). The site will contact the IVRS/IWRS prior to the start of study intervention administration for each participant. The site will record the intervention assignment on the applicable case report form (CRF), if required.

Potential bias will be reduced using a central stratified randomization to assign participants into one of the study treatment arms, RESP301+SOC or SOC (control), with a randomization ratio of 2:1. Participants will be stratified by country and presence of risk factors for severe outcomes of COVID-19, based on comorbidities and age as follows:

1. Age >65 years
2. Ongoing or currently treated diabetes mellitus
3. Ongoing or currently treated hypertension
4. Ongoing or currently treated cardiovascular disease
5. Ongoing or currently treated chronic lung disease
6. Cancer history of less than 3 years, basal cell skin carcinoma excluded
7. Ongoing or currently treated chronic kidney disease

Participants will be stratified as no risk factor (none of the above criteria), one risk factor (one single of the above) or high-risk factor (2 or more of the above).

6.4 Study Intervention Compliance

Participants will receive study intervention directly from the Investigator or designee, under clinical supervision. The date and time of each inhalation will be recorded in the source documents and recorded in the CRF. The study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Concomitant Therapy

During the study, participants will receive institutional SOC for the treatment of COVID-19.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency and route

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1 Potential Drug Interactions

Investigators should be aware of the potential interaction between NO generated by RESP301 and that generated from NO donor agents, such as prilocaine, sodium nitroprusside, and nitroglycerine, that may increase the risk of developing methemoglobinemia.

6.5.2 Rescue Medication

For participants treated before completion of the first safety review by the IDMC (Section 9.6), at the time of RESP301 treatment, a ready-to-use nebulization of a bronchodilator (short acting beta agonist such as salbutamol/albuterol according to investigational site standard practice for acute bronchospasm) may be administered to participants in case of bronchospasm.

After reviewing safety data, the IDMC will decide whether the availability of rescue bronchodilator at the time of RESP301 treatment is still required.

Although the use of rescue medications is allowable for the first 10 participants, the use of rescue medication should be delayed, if possible, for at least 2 minutes following each administration of study intervention. The date and time of rescue medication administration as well as the name of the rescue medication must be recorded.

6.6 Intervention After the End of the Study

After the end of the study, participants may continue their SOC (if any). Study intervention for COVID-19 will not continue beyond this study.

7 Discontinuation of Study Intervention and Participant Discontinuation

7.1 Permanent Discontinuation of Study Intervention

It may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the study to be evaluated for the Day 14 and Day 28 safety follow-ups. See the SoA (Table 1-1) for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

Study intervention will be permanently discontinued prior to 10 days if the participant:

- Improves to level 1 or 2 of the modified WHO ordinal scale;
- Progresses to a level > 4 of the modified WHO ordinal scale.

Additionally, study intervention should be permanently discontinued in the following circumstances:

1. Discontinuation of study intervention for abnormal liver tests should be considered if the Investigator believes that it is in best interest of the participant. The development of alanine aminotransferase (ALT) ≥3 × ULN and bilirubin ≥2 × ULN or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR is measured, which may indicate severe liver injury (according to Hy's Law), must be reported as an SAE and the study intervention discontinued.

2. If, as part of normal clinical follow-up, a clinically significant change in ECG is identified, the Investigators should exercise their clinical judgement to decide whether continuing study intervention administration is in the best interest of safety of the participant.

3. AE/SAE if the Investigator believes that it is in best interest of the participant, including the following:

- Development of significant bronchospasm * or worsening of cough that is not tolerated by the participant and leads to immediate discontinuation of the nebulization.

- Progressive COVID-19 requiring initiation of invasive ventilation or high flow oxygen.

- Development of mHb level above 3%.

- Inability to safely continue receiving study intervention due to compromised respiratory status while off supplemental oxygen and receiving nebulized therapy (manifest by drop in SpO_2, increased respiratory rate or clinical other findings).

Refer to the SoA (Table 1-1) for data to be collected at the time of study intervention discontinuation and follow-up and for any further evaluations that need to be completed.

** Inhaled citric acid and inhaled mannitol provoke bronchospasm in patients with bronchial hyperresponsiveness in a dose dependent pattern. Although the concentrations of mannitol and citric acid are below the provocative dose and concentration inducing bronchospasm, there is a theoretical risk that a participant may develop acute bronchospasm during the use of RESP301. Participants should therefore be monitored during RESP301 administration. In case of clinical intolerance or saturation decrease, nebulization should be immediately discontinued (see Section 7.2). Further clarification of the differences in signs and symptoms for acute bronchospasm and COVID-19 progression are provided in* Table 7-1.

Table 7-1 Differences in Signs and Symptoms for Acute Bronchospasm and COVID-19 Progression

	Acute bronchospasm related to inhaled bronchial irritant	Progression of COVID-19 lung disease to acute respiratory failure
Mechanism	Acute contraction of bronchial muscles resulting in bronchial obstruction resulting in increased ventilatory load	Cytokine storm resulting in alveolar damage resulting in gas transfer impairment
Onset	Within minutes (<15 minutes) following inhalation (no late phase since it is not an allergic IgE mediated reaction)	Within hours or days and not related to nebulization
Clinical auscultatory signs	Wheezing (almost all cases)	Bilateral rales (inconstant)
Patient history	Asthma or respiratory allergy	Age > 65 years, increased BMI, high blood pressure, cardiac diseases, etc.
Reversibility with inhaled beta mimetics	Within minutes	No

Abbreviations: BMI=body mass index; IgE=immunoglobulin E.

7.2 Temporary Discontinuation of Study Intervention

Brief temporary discontinuation of study intervention is permitted during the study, providing inhalation of study intervention is completed within 20 minutes.

Study intervention should be temporarily discontinued in the following circumstance:

1. Clinical intolerance to nebulization.

   An individual administration of study treatment will be stopped, and supplemental oxygen if applicable resumed, if threshold limits of SpO_2 89% or heart rate 120 beats/min (or as indicated by the Investigator) are exceeded (see Sections 4.2 and 8.1.2).

The participant may continue with subsequent doses if the Investigator judges that the benefit / risk ratio remains positive. In this case, the instruction to continue should be duly recorded in the participant's hospital file and the subsequent nebulization should be closely monitored.

Refer to Section 6.1 for information on staggered dosing in the first 10 participants.

7.3 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. The participant will be definitively discontinued from both the study intervention and from the study at that time.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

At the time of discontinuing from the study, if possible, an early discontinuation assessment should be conducted, as shown in the SoA (Table 1-1).

7.4 Loss of Participants to Follow-Up

A participant will be considered lost to follow-up if he or she is unable to be contacted by the study site. The following actions must be taken if a participant cannot be contacted at Day 14 or Day 28:

- The site must attempt to contact the participant and counsel the participant on the importance of maintaining the assigned schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- In cases in which the participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible,

three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record/CRF.

- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 Study Assessments and Procedures

Study procedures and their timing are summarized in the SoA (Table 1-1). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Table 1-1).

The maximum amount of blood collected from each participant, should be approximately 20 mL per occasion (Screen, Day 1 , Day 3, Day 7, Day 10 and Day 28).

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Informed Consent

Informed consent must be documented according to Appendix 1, Section 10.1.3.

Eligibility Criteria

All inclusion and exclusion criteria will be reviewed by the Investigator or designee to ensure that the participant qualifies for the study.

Participant Identification Card

All participants will be given a participant identification card identifying them as participants in a research study. The card will contain study site contact information (including direct telephone numbers) to be used in the event of an emergency. The Investigator or qualified designee will provide the participant with a participant identification card immediately after the participant provides written informed consent. At the time of intervention allocation/randomization, site personnel will add the intervention/randomization number to the participant identification card.

Medical History

A medical history will be obtained by the Investigator or qualified designee. The medical history will collect all active conditions and any condition diagnosed that the Investigator considers to be clinically relevant. Details regarding the disease for which the participant has enrolled in this study will be recorded separately and not listed as medical history.

Prior and Concomitant Medications Review

The Investigator or qualified designee will review prior medication use and record prior medications taken by the participant within 3 months prior to screening. This should include a history of hypertension medication, in particular angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

The Investigator or qualified designee will record medication, if any, taken by the participant during the study through the last assessment. Concomitant medications will be recorded on an ongoing basis during the study (or longer if related to an SAE).

8.1 Efficacy Assessments

8.1.1 Modified WHO Ordinal Scale

A modified WHO ordinal scale will be used for consistency with the recent study of lopinavir-ritonavir in adults hospitalized with severe COVID-19 (Cao et al, 2020), to record the participant's status at the time of assessment. The modified WHO ordinal scale includes the following levels:

1. Not hospitalized, no limitations on activities;
2. Not hospitalized, limitation on activities;
3. Hospitalized, not requiring supplemental oxygen;

4. Hospitalized, requiring supplemental oxygen;
5. Hospitalized, on non-invasive ventilation or high-flow oxygen devices;
6. Hospitalized, on invasive mechanical ventilation or extra corporeal membrane oxygenation (ECMO);
7. Death.

8.1.2 Pulse Oximetry

Pulse oximetry measurements will be performed to evaluate SpO_2 as outlined in the SoA (Table 1-1) and in accordance with the site standard operating procedures, on a medical-grade medical device. Measurements will be taken with a probe on fingertip, toe or earlobe and recorded as percent oxygenated hemoglobin. Supplemental oxygen used at the time of assessment and method of oxygen delivery will be collected and documented along with the SpO_2.

Prior to administration of study intervention, supplemental oxygen will be held for at least a minute and SpO_2 checked in order to ensure participant may safely receive the study intervention. For safety reasons, since due to the current pandemic it cannot be guaranteed that study intervention will be consistently administered under direct clinical supervision, the study Investigators should provide study personnel who are able to monitor the participant and their SpO_2 and ensure that should it decrease to a threshold, this would trigger immediate discontinuation. By default, unless overruled by the study Investigator, this SpO_2 threshold is set to 89%. This threshold should be entered into the saturation monitoring device to trigger an alarm during nebulization.

FiO_2 will be measured per site standard practice and recorded in the eCRF. The device used to administer oxygen should also be recorded.

8.1.3 National Early Warning Score (NEWS) 2

The NEWS 2 will be measured prior to study intervention in the morning according to the SoA in Table 1-1.

The NEWS is based on a simple aggregate scoring system in which a score is allocated to physiological measurements, already recorded in routine practice, when patients present to, or are being monitored in hospital (RCP, 2012). Six simple physiological parameters form the basis of the scoring system:

1. Respiration rate

2. Oxygen saturation
3. Systolic blood pressure
4. Pulse rate
5. Level of consciousness or new confusion*
6. Temperature

**The patient has new-onset confusion, disorientation and/or agitation, where previously their mental state was normal – this may be subtle. The patient may respond to questions coherently, but there is some confusion, disorientation and/or agitation. This would score 3 or 4 on the Glasgow Coma Scale (rather than the normal 5 for verbal response), and scores 3 on the NEWS system.*

A score is allocated to each parameter as they are measured, with the magnitude of the score reflecting how extremely the parameter varies from the norm (zero for 'normal'; maximum score 3). The score is then aggregated. The score is increased by 2 points for people requiring supplemental oxygen to maintain their recommended oxygen saturation. This is a pragmatic approach, with a key emphasis on system-wide standardization and the use of physiological parameters that are already routinely measured in National Health Service (NHS) hospitals and in prehospital care, recorded on a standardized clinical chart – the NEWS 2 chart (Refer to Appendix 2: The NEWS 2 Scoring System).

Reproduced from: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017 (RCP, 2017).

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1-1).

8.2.1 Physical Examinations

A complete physical examination will include, at a minimum, assessments of the head/ear/eyes/nose/throat, cardiovascular, respiratory, gastrointestinal, lymphatic, skin and neurological systems. Height (screening only) and weight will also be measured and recorded.

A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

8.2.2 Vital Signs

Vital signs to be collected as outlined in the SoA (Table 1-1) and include body temperature, heart rate, blood pressure, and respiratory rate.

Body temperature will be assessed per the local practice (temporal or otic are preferred sites), and site will be recorded. Pulse rate, respiratory rate, and blood pressure will also be assessed per site SOC. Where possible the same methods should be used throughout the study for an individual participant.

Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones). During study intervention administration, an alarm threshold should be set for heart rate at 120 beats/min (or as indicated by the Investigator), as outlined in the SoA (Table 1-1).

8.2.3 Electrocardiograms

Single 12-lead ECG will be obtained as outlined in the SoA (Table 1-1) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals for local reading.

8.2.4 Clinical Safety Laboratory Assessments

Refer to Appendix 3 for the list of clinical laboratory tests to be performed and to the SoA (Table 1-1) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically relevant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically abnormal during participation in the study or within 4 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, where possible, and the Sponsor notified.

- All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the Laboratory Manual and the SoA (Table 1-1).
- If laboratory values from laboratory assessments not specified in the protocol and performed at the institution's local laboratory result in the need for a change in participant management or are considered clinically relevant by the Investigator (e.g., are considered to be an SAE or an AE), then the results must be recorded in the CRF.

8.3 Adverse Events and Serious Adverse Events

The definitions of AEs and SAEs can be found in Appendix 4.

Adverse events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or the study, or that caused the participant to discontinue the study intervention (see Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from signing of informed consent until the last follow-up visit at the time points specified in the SoA (Table 1-1).

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours of the Investigator becoming aware of the SAE, as indicated in Appendix 4. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obliged to actively seek AEs or SAEs after the conclusion of study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.3.2 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is given in Appendix 4.

8.3.4 Regulatory Reporting Requirements for SAE

Prompt notification (within 24 hours, see Appendix 4) by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and Investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAE) from the Sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

All women, including women of childbearing potential are allowed in the study. Urine pregnancy test will be performed on female participants of childbearing potential and pregnant females at screening. Females who are not of child bearing potential do not need to undergo a screening pregnancy test.

Pregnant females will be followed to determine the outcome of the pregnancy:

- The Investigator will collect any follow-up information on the participant and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of the pregnancy will be reported, regardless of fetal state (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered to be SAEs. Any post-study pregnancy-related SAE considered reasonably related to study intervention by the Investigator will be reported to the Sponsor as described in Section 8.3.4. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related events (DREs) are common in participants with COVID-19 and can be serious/life-threatening:

- Fever
- Cough*
- Dyspnea*
- Asthenia
- Loss of sense of taste and smell

** A cough or dyspnea episode related to study intervention administration does not meet the definition of a DRE and should be reported as an AE.*

Because these events are typically associated with the disease under study, they will not be reported as AEs.

NOTE: However, if either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the Investigator's opinion, of greater intensity, frequency, or duration than expected for the individual participant.

  OR

- The Investigator considers that there is a reasonable possibility that the event was related to study intervention.

8.3.7 Adverse Events of Special Interest (Not Applicable)

No AEs of special interest are defined for this study.

8.3.8 Medical Device Deficiencies (Not Applicable)

The medical device to be used in this study is a vibrating mesh nebulizer that is FDA-approved and CE-marked and is to be used per the manufacturer's recommendations. Therefore, data on device deficiencies will not be collected in this study.

8.4 Tokenization (Optional)

Tokenization is the process of converting a piece of data into a random string of characters known as a token. Tokenization protects sensitive data by substituting non-sensitive data. The token serves merely as a reference to the original data, but cannot be utilized to determine those values. The advantage of tokens is that there is no mathematical relationship to the real data that they represent. The real data values cannot be obtained through reversal, and hence, a breach renders the information invaluable. Tokens are being increasingly used to secure varying types of sensitive information. In particular, personal identifiable information such as healthcare information, email addresses and account numbers are such examples. From a security perspective, tokenization significantly reduces risk based on the fact that sensitive data cannot be breached if it is not there in the first place.

Tokenization applies only to US participants who agree and sign the optional ICF. The piece of personal data needed to generate the token will be collected to create a unique, de-identified token. This token would be instrumental to enrich and aggregate other study participants' data coming from different sources for the purpose of future research. The benefit of linking data in general, is that the data set that is created can be used to answer a variety of healthcare- and therapy-related questions that could not otherwise be answered through conventional means.

8.5 Treatment of Overdose (Not Applicable)

There is no risk of overdose.

8.6 Pharmacokinetics (Not Applicable)

Pharmacokinetic parameters are not evaluated in this study.

8.7 Pharmacodynamics (Not Applicable)

Pharmacodynamic parameters are not evaluated in this study.

8.8 Genetics (Not Applicable)

Pharmacogenomics are not evaluated in this study.

8.9 Biomarkers (Not Applicable)

Biomarkers are not evaluated in this study.

8.10 Medical Resource Utilization and Health Economics (Not Applicable)

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 Statistical Considerations

9.1 Statistical Hypothesis

RESP301 reduces the rate of progression in the modified WHO ordinal scale in COVID-19 (see Section 8.1.1).

The endpoint of worsening level on the WHO ordinal scale represents a composite endpoint of scales above the entry level, being (i) death (level 7), (ii) hospitalized, on invasive mechanical ventilation or ECMO (level 6), (iii) hospitalized, on non-invasive ventilation or high-flow oxygen devices (level 5) , or, for those patients who were recruited at WHO ordinal scale 3, (iv) hospitalized, requiring supplemental oxygen.

9.2 Sample Size Determination

A total of 300 hospitalized participants with confirmed COVID-19 will be randomized 2:1 to receive RESP301+SOC (200 participants) or SOC alone (100 participants).

The comparison between the treatment arms for the primary endpoint will have approximately 80% power and alpha level 0.025 (one-sided) to demonstrate significant reductions (15% versus 30%) of the primary endpoint between RESP001+SOC and the Control arm (SOC), a 50% relative reduction for the proportion of participants who progress by at least one level on the WHO ordinal scale (see definition of the primary endpoint).

Two interim analyses are planned (see Section 9.5 for details):

1. The first IDMC interim analysis will take place after the first 60 participants have completed Day 10 of the study to evaluate whether the study can be stopped for futility based on change from baseline in room air SpO_2. For a final decision to stop the study for futility the results on other endpoints will be considered as well.
2. The second interim analysis will take place after 150 participants have completed Day 14 post-randomization based on event rate for the primary endpoint. The purpose of the second interim analysis will be futility as well as a potential sample size re-estimation in case the actual results differ from the original assumptions.

In addition to the review of efficacy data, safety will be assessed at each of the interim analysis by the IDMC. Further details are provided in Appendix 5.

9.3 Populations for Analyses

For purposes of analysis, the following analysis sets are defined:

Table 9-1 Populations for Analysis

Population (Analysis Set)	Description
Intent-To-Treat (ITT) Population	The ITT Population will include all randomized participants. The ITT participants will be analyzed according to randomized treatment, irrespective of the actual treatment received. Participants who withdraw from treatment early will be followed for the assessment of the Day 14 primary endpoint. All efficacy analyses will be performed using the ITT Population.
Per Protocol (PP) Population	The PP Population will include all participants in the ITT Population with no major protocol deviations that may significantly impact data integrity or patient safety. The PP Population will be used for supportive analyses of the efficacy measurements.
Safety Population (SP)	The SP will include all randomized participants who inhale any amount of study intervention or are randomized to the control arm. The SP will be analyzed according to the actual treatment received. This set will be used for the safety analyses.

The ITT Population will be the primary analysis set for all efficacy analyses and the PP Population will be used to demonstrate robustness of results for the primary efficacy endpoint.

9.4 Statistical Analyses

Below is a description of planned statistical analyses. Further details are presented in the Statistical Analysis Plan (SAP).

9.4.1 General Considerations

All statistical analyses will be conducted using SAS, Version 9.4 or later. Baseline characteristics will be summarized by treatment arm. For continuous measures the mean and standard deviation (SD) will be summarized. Categorical variables will be described by the proportion in each category. In addition, 95% confidence intervals (CIs) will be computed as indicated.

All the categorical variables including the primary endpoint will be summarized by treatment with the numbers and percentages of the participants. Treatment difference will be tested using a Cochran–Mantel–Haenszel test stratified by 1) country and 2) participant's clinical status at Baseline. For the categorical endpoints, relative risk and its 95% CI will be presented.

All of the continuous variables, including the changes from Baseline, will be summarized by treatment with the means, SD, medians and the ranges. The mixed model with repeated measurements /analysis of covariance model with treatment, country, participant's clinical status at Baseline and visit as the model term, and Baseline value as the covariate will be used to test for the significance of the treatment difference. Least square means, standard errors, 95% CIs and p-values will be presented.

Time to event endpoints will be analyzed using the Cox Proportional Hazard model with treatment, participant's clinical status at Baseline and country as the model term. The hazard ratio of RESP301+SOC versus SOC will be presented along with 95% CI and p-value from the model. The Kaplan-Meier curves of the time to events will be presented by treatment for each applicable endpoint.

Handling of missing data

If participants are discharged from the hospital prior to Day 14 due to improvement of the clinical status and their status on Day 14 cannot be obtained, their status on Day 14 for the primary endpoint will be imputed with the status on the day of discharge. Depending on the reasons for missing data on the primary endpoint up to Day 14, additional sensitivity analyses will be performed. Further details on handling on missing data will be provided in the SAP.

9.4.2 Primary Endpoint

The primary endpoint is the proportion of participants who progress by at least one level higher on the modified WHO ordinal scale by Day 14.

9.4.3 Secondary Endpoint(s)

The key secondary endpoints are:

1. Change from baseline on the modified WHO ordinal scale at each visit up to Day 28
2. Change in room air SpO_2 from baseline over time
3. Change in NEWS 2 symptom score from baseline over time
4. Time to improvement of at least one level lower on the modified WHO ordinal scale
5. Time to progression of at least one level higher on the modified WHO ordinal scale

Additional secondary endpoints are:

1. Time to hospital discharge
2. Incidence of mortality by Day 28

9.4.4 Tertiary/Exploratory Endpoint(s)

CCI

- CCI

9.4.5 Other Safety Analyses

All safety analyses will be performed on the Safety Population.

1. Safety and tolerability assessed by clinical safety laboratory measurements, physical examinations, vital signs, concomitant medications; cumulative incidence of AEs, SAEs and severe AEs
2. Incidence of participants unable to tolerate nebulization due to:
    - Reduction in SpO_2 to < 90%, unless well clinically tolerated according to Investigator's opinion
    - Other clinical signs of intolerance according to Investigator's opinion
3. Incidence of clinical bronchial hyper-responsiveness related to nebulization

9.4.5.1 Adverse Events

Adverse Events will be coded using the MedDRA coding dictionary.

The number and percentage of participants with any AE, any related AE, any SAE, any related SAE, any severe AE, and related severe AE as well as the total number of events for each category will be summarized. The number of deaths due to an AE, hospitalization due to an AE, and study discontinuation due to an AE will be summarized.

The number and percentage of participants with an AE, as well as the total number of AEs, will be summarized by SOC and preferred term. This tabulation will be repeated for related AEs, SAEs, related SAEs, severe AEs, and related severe AEs.

All AEs will be provided in patient listings. Patient listings of AEs causing discontinuation of study medication, AEs leading to death, SAEs, related AEs, and severe AEs will be produced.

9.4.5.2 Clinical Laboratory Evaluation

Baseline is defined as the last non-missing value obtained at the screening visit and prior to the first exposure to study drug. Actual values and changes from Baseline clinical laboratory tests will be summarized by study day. If more than one laboratory result is reported per study visit per parameter, the last non-missing result will be selected for change from Baseline analysis.

Laboratory test results will be classified according to the reference ranges and clinical significance as determined by the Investigator. The number of participants with a non-missing result, the number and percentage of participants with a clinically significant result less than the lower limit of normal, non-clinically significant result more than the ULN, and clinically significant result more than the ULN will be summarized by study visit. If more than one laboratory result is reported per study day per parameter, the result yielding the most severe classification will be selected for analysis.

Categorical laboratory test results (urinalysis excluding pH) will be summarized by study visit. If more than one laboratory result is reported per study day per parameters, the result yielding the most severe classification will be selected for analysis.

Participants who had urine pregnancy test at screening and the results will be listed.

Participants with clinically significant abnormal laboratory test results will be listed. This listing will include all results of the laboratory parameter that was abnormal and determined to be clinically significant by the Investigator for a participant across study visit.

9.4.5.3 Vital Signs

Baseline is defined as the last non-missing value obtained in screening and prior to the first exposure to study drug. Actual values and changes from Baseline in vital signs will be summarized by study day and study time point. All vital sign data will be presented in patient listings.

Vital sign values will be classified according to the clinical significance as determined by the Investigator. The number of participants with a non-missing result, the number and percentage of participants with a non-clinically significant result, and clinically significant result will be summarized by study visit and study time point. If more than one vital sign result is reported per study day and study time point per parameter, the result yielding the most severe classification will be selected for analysis.

Participants with clinically significant vital sign values will be listed. This listing will include all results of the vital sign parameter that was determined by the Investigator to be clinically significant for a participant across study time points.

9.4.5.4 Physical Examination

Abnormal physical examination findings will be listed.

9.4.6 Other Analyses

Other analyses may be added to the SAP as applicable.

9.5 Interim Analyses

Two interim analyses will be performed.

1. The first analysis will be conducted when about 60 participants (40 participants in the RESP301+SOC arm and 20 participants in the SOC arm) have completed the Day 10 assessment. The purpose of this analysis is to evaluate efficacy of RESP301 with the application of a futility criterion based on the results on SpO_2 change from baseline on Day 10. The following futility criterion will be used for this first interim analysis:

   If the difference in the percentage of participants with at least a 2% improvement in SpO_2 between both treatment arms is less than 5%, benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

As no other modification of the study at the time of the first interim analysis is considered, no adjustment of the alpha level is required.

2. The second interim analysis will be conducted after 150 participants (100 participants in the RESP301+SOC arm and 50 participants in the SOC arm) have completed the Day 14 assessment. The purpose of this analysis is the assessment of futility or a sample size re-estimation in case the actual results differ from the original assumptions for the power calculations of the study, related to the percentage of participants meeting the primary endpoint in the control arm and/or the relative treatment benefit achieved in the RESP301+SOC arm compared to the SOC arm.

   To account for the multiple testing due to the second interim analysis an adjustment for the type I error alpha will be applied using the Haybittle-Peto approach which would spend one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis. The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration will be given to stop the study for futility. At the same time sample size re-estimation will be performed to achieve 80% power at the end of the study. The methodology for the adjustment and the procedure to maintain the type I error level will be described in greater detail in the SAP.

Detailed information, including the boundaries futility and characteristics for the sample size re-estimation at the time of the interim analyses will be provided in the SAP and Data Monitoring Committee (DMC) Charter. Further details are also provided in Appendix 5.

9.6 Data Monitoring Committee (DMC)

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study. The IDMC will meet to review after 10 and 20 participants have completed as well as at the time of the interim analyses (i.e., after 60 and 150 participants have completed; see Section 9.5).

Due to the urgency of the pandemic, if participants become eligible for the study during the IDMC review of the first 10 participants then they may be recruited. However, their administration of RESP301 will be staggered in the same way as for the first 10 participants (see also Section 6.1). Once the IDMC review has been completed, and provided there are no safety concerns, further recruitment will continue as per protocol. The Sponsor and CRO confirm that

the report from the IDMC will be sent in a timely manner to all participating sites and investigators.

The detailed procedures and criteria of the interim analyses will be described in the DMC Charter.

10 Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
    - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
    - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The Investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC.
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures.
    - Overall conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH GCP guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Financial Disclosure

Information on financial disclosure can be found in the Investigator Site File.

10.1.3 Informed Consent Process

The Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IEC/IRB and signed by all participants subsequently enrolled in the study as well as those currently enrolled in the study.

For US participants who agree to tokenization, a separate optional ICF will be provided.

10.1.4 Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Data Protection for Tokenization information is addressed in the separate optional ICF (for US participants only).

10.1.5 Committees Structure

An IDMC will be responsible for closely reviewing the safety and efficacy data from the interim analyses and for providing their recommendations on continuation of the study (see Sections 9.5 and 9.6).

10.1.6 Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategies (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations [CROs]).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for at least 2 years after the last approval of a marketing application or 15 years from completion of the study, whichever is longer according to the relevant local laws and/or regulations. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

- All data generated by the site personnel will be captured electronically at each study center using eCRFs. Data from external sources (such as laboratory data) will be imported into the database. Once the eCRF clinical data have been submitted to the central server at the independent data center, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.
- If additional corrections are needed, the responsible monitor or data manager will raise a query in the electronic data capture (EDC) application. The appropriate staff at the study site will answer queries sent to the Investigator. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will freeze the eCRF page.
- The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the EDC system/eCRF.

10.1.7 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

10.1.8 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the first participant signing the informed consent form and will be the study start date.

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the Investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up

10.1.9 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor at least one month before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.10 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first participant is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/Competent Authority approval prior to implementation (if appropriate). In the US: Following approval, the protocol

amendment(s) will be submitted to the Investigational New Drug application under which the study is being conducted.

Administrative changes (not affecting the participant benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.1.11 Liability and Insurance

10.1.11.1 Access to Source Data

Access to source data is described in the Clinical Site contract.

10.2 Appendix 2: The NEWS 2 Scoring System

Chart 1: The NEWS 2 scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

Abbreviations: CVPU: C=new onset confusion, disorientation or agitation, V=responds to voice, P=responds to pain, U=unresponsive; SpO_2=oxygen saturation.

Source: Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP, 2017. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2

10.3 Appendix 3: Clinical Laboratory Tests

The tests detailed in Table 1-1 will be performed by the local laboratory.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol. Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 10-1 Protocol-Required Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	CBC without differential: White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet, mean platelet volume Methemoglobin, nitrite			
Clinical Chemistry[1]	Blood urea nitrogen Creatinine Glucose non-fasting	Potassium Sodium Chloride	Aspartate Aminotransferase Alanine Aminotransferase/ Alkaline phosphatase Lactate dehydrogenase	Total bilirubin Coagulation (PT/INR, aPTT)
Other Screening Tests	Highly sensitive urine human chorionic gonadotropin pregnancy test (for women of childbearing potential and pregnant women) [2] The results of each test must be entered into the eCRF.			
NOTES: 1 Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1. All events of ALT ≥3 × ULN and bilirubin ≥2 × ULN or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if				

Laboratory Assessments	Parameters
INR is measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE and the study intervention discontinued. 2 Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.	

Investigators must document their review of each laboratory safety report.

10.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1 Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (e.g., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.4.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization (Applies only during the safety follow up period for participants who may have been discharged during the treatment period, i.e., between Day 1 and Day 10)

- In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or intervention that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are considered AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective intervention of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive intervention in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.4.3 Recording and Follow-up of AE and SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event. • The Investigator will then record all relevant AE/SAE information in the CRF. • It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to the clinical research organization (CRO) in lieu of completion of the AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by the CRO. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to Parexel Safety Services for review. • The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories: • Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities. • Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe. An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report in the electronic CRF/EDC. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to electronic CRF/EDC.**
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the DMC to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed CRF.

- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.4.4 Reporting of SAE

SAE Reporting to Parexel Safety Services via Electronic Data Collection Tool

The Investigator must report any SAEs to the Parexel Safety Services within 24 hours of becoming aware of the event.

All SAEs will be recorded from signing of informed consent until the end of the study. Serious adverse events occurring after the end of the study and coming to the attention of the Investigator must be reported only if they are considered (in the opinion of the Investigator) causally related to study intervention.

- The primary mechanism for reporting an SAE to Parexel Safety Services will be the electronic data collection tool.
- The site will additionally use the paper SAE data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- The Investigator and the Sponsor (or Sponsor's designated agent) will review each SAE report and the Sponsor/Parexel will evaluate the seriousness and the causal relationship of the event to study intervention. In addition, the Sponsor (or Sponsor's designated agent) will evaluate the expectedness according to the reference document (Investigator Brochure or Summary of Product Characteristics). Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by email.
- Contacts for SAE reporting can be found in the Investigator Manual.

Suspected Unexpected Serious Adverse Reactions (SUSARs)
Any AE that is a SUSAR has additional reporting requirements, as described below. • If the SUSAR is fatal or life-threatening, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within seven calendar days after the Sponsor learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information should be reported within an additional 8 days (15 days total). • If the SUSAR is not fatal or life-threatening but is otherwise serious, associated with study intervention, and unexpected, regulatory authorities and IECs will be notified within 15 calendar days after the Sponsor learns of the event. The Sponsor will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of participants. Follow-up information may be submitted if necessary. The Sponsor will also provide annual safety updates to the regulatory authorities and IECs responsible for the study. These updates will include information on SUSARs and other relevant safety findings.

All SAEs that occur during the study, and all SAEs occurring up to 18 days after receiving the last dose of study intervention, whether considered to be associated with the study intervention or not, must be reported within 24 hours via electronic data collection tool and paper data collection tool to Parexel Safety Services.

The minimum information required for an initial report is:

Name of person sending the report (e.g., name, address of Investigator);

- Participant identification (screening/randomization number, initials, NOT participant name);
- Protocol number;
- Description of SAE;
- Causality assessment, if possible.

However, as far as possible all points on the SAE form should be covered in the initial report, or the completed SAE form itself must be emailed or faxed to the Parexel Safety Services. In addition, the event must be documented in the electronic CRF/EDC system.

After receipt of the initial report, Parexel Safety Services will review the information and, if necessary, contact the Investigator, to obtain further information for assessment of the event. Parexel will be responsible for all information processing and reporting according to local legal requirements. Where necessary, Investigators will inform regulatory authorities in their own countries.

10.5 Appendix 5: Statistical Design Considerations

10.5.1 Some General Design Considerations

1. Binary primary endpoint: progression to critical stage/death up to Day 14
    - to be analyzed by a Cochran-Mantel-Haenszel (CMH) stratified for the factors used in the stratified randomization
    - subjects who withdraw from study treatment will be followed up until their primary endpoint outcome is known (i.e., up to Day 14 or progression to critical stage/death, whatever is first)
    - it is, therefore, not clear what current protocol synopsis text could mean: *If subjects are discharged from the hospital prior to day 14 due to improvement of the clinical status and their status on day 14 cannot be obtained, their status on day 14 for the primary endpoint will be imputed with the status on the day of discharge. and what the implications on statistical study characteristics could be. Further discussion is needed.*
2. Timing of the two planned interim analyses with stopping options is stated as

There are 2 interim analyses planned, the first interim analysis after the first 60 participants have completed day 10 of the observation period … and a second interim analysis after 150 participants have completed day 14 of the observation period.

With the current text for the timing of the first interim analysis, it is not clear how many subjects will have been randomized at least 14 days prior to the interim data cut-off date and can therefore provide data for the binary primary endpoint ("progression to critical stage/death up to Day 14").

For such a binary endpoint, Parexel does not recommend including any participant randomized less than 14 days prior to the interim data cut-off date (note this would be different to a study with a time-to-event endpoint) as

- for such participants not yet progressed, the outcome up to Day 14 is unknown and should not be imputed as "not progressed up to Day 14"
- for such participants progressed prior to Day 14, the outcome is known but their inclusion would bias the estimation of progression probability up to Day 14 in an upwards direction.

In order to set up the adaptive group-sequential study design, the following assumptions have been used:

- first interim analysis: a total of ≈ 39 participants randomized at least 14 days prior to data cut-off date
- second interim analysis: a total of ≈ 50% of the initially planned number of participants randomized at least 14 days prior to data cut-off date.

3. Non-binding DMC guidance for stopping the study for futility at the first interim analysis is stated as:

If the difference in the percentage of participants with at least a 2% improvement in SpO_2 between both treatment arms is less than 5%, a benefit of RESP301 treatment is not expected. For a final decision to stop the study for futility the results on other endpoints will be considered as well.

As the main futility stopping criterion is based on improvement in SpO_2 (and not on the primary efficacy endpoint), this first interim analysis will be "ignored" in these statistical considerations for an adaptive group-sequential design. We may revisit the first interim analysis at a later point in time when more information is available.

4. Non-binding DMC guidance for stopping the study for futility at the second interim analysis stated as:

The futility criterion for the second interim analysis will be based on the conditional power (CP) for the final results based on the assumption that the remainder of the study will follow the same trend as observed in the interim analysis. If CP at the time of the second interim analysis is less than 20% consideration will be given to stop the study for futility.

5. Current DMC guidance for stopping the study for superior efficacy at the second interim analysis is stated as:

To account for the multiple testing due to the second interim analysis an adjustment for the type-I-error probability will be applied using the Haybittle-Peto approach with one sided alpha=0.0005 at the second interim analysis and leave one-sided nominal alpha of 0.0249 for the final analysis.

6. For initial sample size calculation, the following additional assumptions / features from the protocol have been used

- progression probability up to Day 14 for Standard of Care (SOC): 30%

- progression probability up to Day 14 for RESP301+SOC: 15%
- desired power assuming the alternative hypothesis stated above: 80%
- 2:1 randomization

7. Method for adaptation of the sample size based on the second interim analysis data is stated as

A sample size re-estimation will be performed to achieve 80% conditional power at the end of the study. The methodology for the adjustment and the procedure to maintain the type-I-error level will be described in greater detail in an appendix to the protocol. The maximum increase of the sample size will be limited to a total number of 600 randomized subjects.

Parexel has put a suggestions with some fictitious interim data for illustration in Section 10.5.3; that section also illustrates by simulations the Cui/Hung/Wang (CWH) approach using a weighted (weights fixed at the design-stage) test for the final analysis versus the Chen/DeMets/Lan (CDL) approach without such weights at the final analysis.

10.5.2 General Design Characteristics

Parexel proposes to base non-binding futility stopping criteria on conditional power criteria. Conditional power (CP) is the conditional probability for achieving statistically significant superiority of RESP301+SOC over SOC at the final analysis, given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study (an alternative would be predictive power (PP), a Bayesian version of CP where prior distributions and observed interim results are combined to obtain the probability for achieving statistically significant superiority of gimsilumab over placebo at the final analysis).

Randomization 2:1	**Group-Sequential Design**
1st Interim Analysis (IA) 2nd IA	N ≈ 39 for D14 ≈ 50% for D14
Futility criterion 1st IA Futility criterion 2nd IA Superior efficacy criterion 2nd IA	Based on SpO_2 CP < 4% p < 0.0005
Total sample size required for 80% power	**300**
Futility stopping probability at 2nd IA under "No effect" Futility stopping probability at 2nd IA under "Planned effect 30% vs 15%"	1st IA: **NA** 2nd IA: **70%** 1st IA: **NA** 2nd IA: **6%**
Superiority stopping probability at 2nd IA under "Planned effect 30% vs 15%" Superiority stopping probability at 2nd IA under "30% vs 10%"	**10%** **28%**

10.5.3 Adaptive Sample Size Re-Estimation

Another objective of the 2nd interim analysis is to re-estimate the sample size based on the unblinded interim results.

Proposed procedure at 2nd interim analysis (planned to include approximately 150 subjects)

- unblinded analysis of the primary efficacy endpoint ("Proportion of participants who progress by at least one level higher on the modified WHO ordinal scale by Day 14" as a binary endpoint)
- calculation of conditional power (CP) given the interim results and calculated by assuming the interim estimates to be the true distribution parameters for the remaining part of the study
    - if CP < 4%: recommend early stop for futility
    - if one-sided p-value < 0.0005: recommend early stop for superior efficacy
    - if 50% < CP < 80% and one-sided p-value ≥ 0.0005:
        - continue the study with an increased total sample size N*, so that CP with a total of N* subjects is increased to 80%, same value as the (unconditional) desired power in the study design
        - N*, however, is limited to 600 (which is twice the original sample size 300)
    - if 4% ≤ CP ≤ 50%: continue the study without a change in sample size (otherwise, the sample size would need to be increased too much or the maximum sample size of 600 would not be sufficient to come close to a CP of 80%.

The table on the next page illustrated the proposed procedure for a number of possible results observed at the 2nd interim analysis.

Table with examples for the adaptive sample size procedure described above using the Cui/Hung/Wang (CHW) approach using a weighted (fixed weights determined by the stage-wise sample sizes planned at the design-stage) test for the final analysis:

2nd IA results with n=150 subjects (50 for SOC, 100 for RESP301+SOC		**CP under observed trend for planned 300**	**Total sample size required for CP 80% under observed trend**	**CP under observed trend if total sample size capped by 600**
15 (30%)	5 (5%)	$p < 0.0005$ → early stop for superior efficacy		
15 (30%)	10 (10%)	99%	Not applicable	Not applicable
15 (30%)	15 (15%)	90%	Not applicable	Not applicable
15 (30%)	18 (18%)	66%	393	Not applicable
15 (30%)	19 (19%)	55%	483	Not applicable
15 (30%)	21 (21%)	34%	[798]	[67%]
15 (30%)	26 (26%)	3.99%	Early stop for futility	
12 (24%)	10 (10%)	92%	Not applicable	Not applicable
12 (24%)	12 (12%)	77%	321	Not applicable
12 (24%)	14 (14%)	54%	498	Not applicable
12 (24%)	15 (15%)	42%	[646]	[77%]
12 (24%)	18 (18%)	14%	[1800]	[31%]
12 (24%)	20 (20%)	2.6%	Early stop for futility	
10 (20%)	8 (8%)	85%	Not applicable	Not applicable
10 (20%)	10 (10%)	64%	412	Not applicable
10 (20%)	11 (11%)	50.4%	534	Not applicable

10 (20%)	13 (13%)	26%	[1014]	[55%]
10 (20%)	15 (15%)	10%	[2460]	[23%]
10 (20%)	18 (18%)	1.4%	Early stop for futility	

Quantities in [ ] indicate theoretical values as sample size would not be increased per the currently proposed adaptive design.

10.5.4 Overall Adaptive Design Performance

Finally, the Cui/Hung/Wang (CHW) approach is compared to the Chen/DeMets/Lan (CDL) approach without pre-defined fixed weights for the final analysis (which is valid as sample size may only be increased if CP (obtained under observed trend) at the second interim analysis exceeds 50%).

All the results below are for the group-sequential design with adaptive sample size re-estimation procedure as described in previous sections and based on 10.000 simulated trials each.

Scenario 1: true progression probabilities are 30% (SOC) and 15% (RESP301+SOC)

Scenario 2: true progression probabilities are 24% (SOC) and 14% (RESP301+SOC)

Scenario 3: true progression probabilities are 30% (SOC) and 10% (RESP301+SOC)

Scenario 4: true progression probabilities are 24% (SOC) and 20% (RESP301+SOC)

10.6 Appendix 6: Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CBC	complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CP	Conditional power
CRF	case report form(s) (paper or electronic as appropriate for this study)
CRO	contract research organization
CV	cardiovascular
DMC	Data Monitoring Committee
DRE	disease-related events
ECG	electrocardiogram
ECMO	Extra corporeal membrane oxygenation
EDC	electronic data capture
EoS	End of study
FDA	Food and Drug Administration
FiO_2	fraction of inspired oxygen
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IVRS	interactive voice response system

IWRS	interactive web response system
LFT	Liver function test
mHb	Methemoglobin
NEWS	National Early Warning Score
NHS	National Health Service
NO	Nitric oxide
NO_2	Nitrogen dioxide
NOx	Oxide of nitrogen
RT-PCR	Reverse transcriptase polymerase chain reaction
PP	Per protocol
PT	Prothrombin time
QTcF	QT interval corrected using Fridericia's formula
SAE	serious adverse event
SAP	statistical analysis plan
SD	Standard deviation
SoA	schedule of activities
SOC	Standard of care
SP	Safety population
SpO_2	Oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
TID	Three times daily
ULN	Upper limit of normal
US	United States
WHO	World Health Organization

10.7 Appendix 7: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

10.7.1 Protocol Amendment 1.0, 05 Jun 2020

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the EU.

Overall Rationale for the Amendment:

The original protocol was updated to include important safety guidelines for study intervention administration for the first 10 participants (i.e., up to the first independent data monitoring committee [IDMC] safety review). It was also updated to align with the IB Version 2.0, dated 27 May 2020. Other changes, with brief rationale, are summarized in the following table.

Section # and Name	Description of Change	Brief Rationale
Global change	Device name Philips InnoSpire Go was replaced with generic reference to a vibrating mesh nebulizer.	The decision was made to keep the device generic for flexibility.
Section 1.1 Synopsis and Section 4.1 Overall Design	Clarified that participants were (male or female) adults. Wording of study design also clarified to include study intervention and minor clarification to text for screening period.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 1.1 Synopsis, Section 4.1 Overall Design, Section 7.1, Discontinuation of Study Intervention	Bullets specifying reasons for permanent discontinuation updated to reflect content of Section 7.1.	Correction, for consistency across the protocol.

Section # and Name	Description of Change	Brief Rationale
Section 1.2 Schema	Dose of study intervention corrected to 6.0 mL. Minor clarifications added in footnotes.	Correction.
Section 1.2 Schema	Footnote 2 updated.	Text added to make it clear that participants will be allowed sufficient time to consider their participation in the study.
Section 1.1 Synopsis, Section 1.3 Schedule of Activities (SoA), Section 2.2.1 Risk Assessment, Section 4.1 Overall Design, and Section 6.1 Study Intervention Administered	The following statement was added: A staggered dosing approach will be used for the first 10 participants (up to the first IDMC safety review). RESP301 will be administered by intermittent inhalation to monitor the tolerability and response to RESP301 (see Sections 6.1 and 9.6). The time gap between consecutive doses in the 3 times per day (TID) schedule was updated to “at least 6 hours” from “at least 4 hours”.	Updated to align with the Investigator Brochure (IB) Version 2.0, dated 27 May 2020.
Section 1.3 SoA, Section 5.2 Inclusion Criteria, Section 8.3.5 Pregnancy, Appendix 3, Clinical Laboratory Tests	Addition of urine pregnancy test at screening. Requirement for all females of childbearing potential, including pregnant females, to consent to urine pregnancy testing at screening. Details of pregnancy follow-up procedures added.	All women, including women of childbearing potential and pregnant women, are allowed in the study. Therefore, it is necessary to determine pregnancy status at study entry so that pregnant females can be followed to determine the outcome of the pregnancy.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 SoA, Appendix 3, Clinical Laboratory Tests	Deletion of tuberculosis (TB) screening test. Nitrite added to the protocol-required laboratory assessments (End of Study [EoS] only).	Screening TB test is not mandatory for this study. Nitrite is to be measured for participants who are still hospitalized at EoS.
Section 1.3 SoA and Section 7.1 Permanent discontinuation of Study Intervention	Deletion of electrocardiogram (ECG) testing after screening. Clarified that significant changes in ECGs would be identified only as part of normal clinical follow-up.	Only routine ECG monitoring is needed for this study.
Section 2.1 Background, Section 11 References	Text deleted from end of paragraph 12. References no longer cited were removed from Section 11.	Removal of text that was specific to the Philips InnoSpire Go nebulizer.
Table 2-1, Risk Assessment and Section 11 References	The risk assessment table was updated to align with the above changes and IB. A new reference (Brannan et al, 2005) was added to Section 11 to support additional text around risk of bronchospasm.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 1.1 Synopsis, Section 1.3 SoA, Section 4.1, Overall Design	Visit window for Day 14 and Day 28 follow-up extended to (± 2 days).	To allow more flexibility.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 SoA	Footnote 2 corrected to include weight and exclude ECG. Clarified that laboratory testing at EoS follow up is for nitrite and methemoglobin only.	Correction and clarification.
Section 1.3 SoA	Footnote 6e corrected from 'Recording of SpO_2 * pre-nebulization on ambient air' to 'Recording of SpO_2 * on ambient air'.	'pre-nebulization' was removed as SpO_2 measurement on ambient air is required for both active and control group.
Section 1.3 SoA	Footnote 7 amended to clarify text that is applicable to participants in the active arm only.	Clarification.
Section 1.3 SoA, Section 8.3 Adverse Events and Serious Adverse Events, Section 8.3.8 Medical Device Deficiencies, and Appendix 5: Medical Device Adverse Events (AEs) Adverse Device Effects (ADEs), Serious Adverse Events (SAEs) and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-up, and Reporting	Changes related to the removal of Appendix 5. Table 8-1 deleted.	Appendix and table were not required because the nebulizer device used in the study is licensed and there is no requirement to collect data on the device.

Section # and Name	Description of Change	Brief Rationale
Section 2.1 Background	Updated to include a description of the three mechanisms of action of RESP301 in fighting virus infection, including addition of new figure (Figure 2–1).	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 2.2.2 Benefit Assessment	Clarification added regarding choice of nebulizer for this study.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Section 3 Objectives and Endpoints	Safety endpoint for AEs amended to include both counts and cumulative incidence.	Both types of data will be summarized.
Section 3 Objectives and Endpoints, Section 9.4.4 Tertiary/Exploratory Endpoint(s)	Exploratory endpoint: timepoint for change from baseline measurement of fraction of inspired oxygen (FiO_2) changed from Day 7 to Day 10.	Correction; the endpoint should be measured at end of treatment.
Section 4.4 End of Study Definition	The following clarification was added to the definition: "End of the study is defined as the last participant's last visit or follow up call."	Clarification of definition.

Section # and Name	Description of Change	Brief Rationale
Section 6.1 Study Intervention(s) Administered	This section (including Table 6-1) was updated to include dosing instructions for the first 10 participants (i.e., up to the first IDMC safety review). In the event of bronchospasm, rescue bronchodilator can be initiated if needed. A new figure was also added to clearly show the staggered approach (Figure 6-1). The TID dosage was also clarified as every 8 hours with at least 6 hours between consecutive doses. Dosing Instructions were updated to clarify that nasal oxygen may be administered during nebulization if the participant is unable to tolerate nebulization without supplemental oxygen.	Specific study drug administration guidelines (including the use of a rescue medication) were provided to monitor tolerability and response to RESP301 inhalation (as described in the risk-benefit table in Section 2.2.1) and to align with the IB, Version 2.0, dated 27 May 2020.
Section 6.2.1 Preparation of Study Intervention Product	Instructions for mixing the two solutions were updated. Further clarification on the delivered dose was added.	Updated to align with the IB Version 2.0, dated 27 May 2020.

Section # and Name	Description of Change	Brief Rationale
Section 6.5.1 Rescue Medication	A new section was added to describe allowed bronchodilator rescue medication for participants treated before the first IDMC safety review (short acting beta agonist such as salbutamol/ albuterol according to investigational site standard practice for acute bronchospasm)	Allowed rescue medication was added to minimize the risk of bronchospasm to participants, and to align with the IB Version 2.0, dated 27 May 2020.
Section 7.1 Discontinuation of Study Intervention	Rationale was added for additional monitoring and discontinuation guidance in case of bronchospasm. Table 7-1 added to provide additional clarification on the differences in signs and symptoms for acute bronchospasm and COVID-19 progression.	Safety guidance (including the use of a rescue medication) was updated to minimize the risk of bronchospasm to participants (as described in the risk-benefit table in Section 2.2.1) and to align with the IB Version 2.0, dated 27 May 2020.
Section 7.1 Discontinuation of Study Intervention Section 7.2, Temporary Discontinuation of Study Intervention Subsequent subsections of renumbered to account for new Section 7.2.	Heading updated to "Permanent" Discontinuation of Study Intervention. Text relating to temporary discontinuation moved to new Section 7.2.	Text describing temporary discontinuation now moved into separate section for clarity and consistency.

Section # and Name	Description of Change	Brief Rationale
Section 8.3.1 Time Period and Frequency for Collecting AE and SAE Information	Corrected statement that AEs and SAEs will be collected from signing of informed consent and clarified that SAEs will be reported within 24 hours of the Investigator becoming aware of the SAE, to align with Appendix 4 (previously it stated 'from randomization'). Deleted second paragraph.	Internal document consistency.
Section 8.3.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs	Loss of sense of taste and smell added to the list of common DREs in participants with COVID-19.	Updated in line with updated World Health Guidelines.
Section 9.2 Sample Size Determination, Section 9.5 Interim Analyses, and Appendix 5, Section 10.5.1 Some General Design Considerations	The timing of the interim analysis was changed from when 60 participants have completed the Day 7 assessment to when 60 participants have completed the Day 10 assessment to make it consistent with the planned treatment duration and the SoA.	Internal document consistency.
Section 9.4.5.2 Clinical Laboratory Evaluation	The following sentence was added: Participants who had urine pregnancy test at screening and the results will be listed.	Updated to include pregnancy test data collection.

Section # and Name	Description of Change	Brief Rationale
Appendix 1, Section 10.1.3 Informed Consent Process, and Section 10.1.4 Data Protection	Text added clarifying that a separate optional ICF will be provided for US participants who agree to tokenization.	Added clarification that tokenization is for US participants only.
Appendix 4, Section 10.4.3 Recording of follow-up of AE and SAE, Section 10.4.4 Reporting of SAE	Alignment of reporting details to state 'Parexel Safety Services' throughout appendix. Text was added to include paper SAE data collection tool.	Internal document consistency and inclusion of paper SAE reporting data collection tool.
Appendix 7, Abbreviations	Heading number updated to reflect deletion of Appendix 6. Updated to reflect changes to abbreviations used in the document.	Consistency.
Section 11 References	Five new references were added to support the above updates (Basu et al, Benz et al, Brannen et al, Colosanti et al, and Saura et al). Two references were deleted as they are no longer cited.	Updated to align with the IB Version 2.0, dated 27 May 2020.
Whole document	Minor language and format changes.	For improved clarity and readability.

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Investigator Agreement Page

Declaration of the Principal or Global Coordinating Investigator

Title: **An open-label, adaptive randomized, controlled multicenter study to evaluate the efficacy and safety of RESP301 plus standard of care (SOC) compared to SOC alone in hospitalized participants with COVID-19 WHO grade 3&4 (NOCoV2)**

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the *Declaration of Helsinki* and the guidelines on Good Clinical Practice.

Principal or Global Coordinating Investigator

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Signature Date

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Name (block letters)

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Title (block letters)

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Institution (block letters)

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Phone number

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Investigator Agreement Page

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Principal or Global Coordinating Investigator

PPD